EFFECTS OF AN 8-DAY FIELD TRAINING ON BODY COMPOSITION, SERUM HORMONE CONCENTRATIONS AND MAXIMAL FORCE PRODUCTION IN MILITARY CONSCRIPTS

Sini Savolainen

University of Jyväskylä
Department of Biology of Physical Activity
Master Thesis
Science of Sports Coaching and Fitness Testing
Spring 2015
Supervisors: Heikki Kyröläinen and Keijo Häkkinen

ABSTRACT

Savolainen, S. 2015. Effects of an 8-day field training on body composition, serum hormone concentrations and maximal force production in military conscripts, 64p. University of Jyväskylä. Department of Biology of Physical Activity. Science of Sports Coaching and Fitness Testing.

The aim of this study was to examine physiological consequences on 8-day strenuous military field exercise. 26 men (20 ± 1 yrs, 53,7 ± 4,5ml/kg/min, 178,4 ± 7,2cm, 71,2 ± 7,2kg) in the end of their military service participated in the study. The research data was collected in five testing day (pre1, pre2, middle, post and follow-up); body composition, physical performance and basal blood levels were measured. The changes in body composition were calculated with the information from total body water measurements with deuterium dilution techique. Serum hormones analysed from the basal blood were total testosterone and cortisol and carrier protein SHBG. Bilateral isometric leg and arm extension force were used to describe physical performance. RESULTS: Total body mass decreased by 1.8±1kg ($p<0.001$) during the 8-day field exercise. Fat mass absolute decrease was 2.0±1kg ($p<0.001$) and a relative change was -18.7±13 %. The absolute change in total fat percentage was -2.4±2% ($p<0.001$). Maximum voluntary contraction decreased during the field exercise in bench press and regained in the end of the field exercise; 761±131 N (pre1); 758±127 N (pre2); 676±137 N (middle, $p<0.001$); 727±117 N (post, $p<0.05$) measurements. Relative change between pre2 and middle measurements is 11%. Maximum voluntary contraction decreased in leg press also and regained in the end of field exercise; 2560±512 N (pre1); 2592±520 N (pre2); 2401±584 N (middle, $p<0.01$); 2499±541 N (post, $p<0.01$). Relative change in maximum voluntary contraction between pre2 and middle measurements is 7%. Significances compared to pre2 measurement. Maximal rate of force development in the bench press was 24492±12919 N/s (pre1); 22865±13253 N/s (pre2); 15564±7397 N/s (middle, $p<0.01$); 15942±7276 N/s (post $p<0.01$). Maximal rate of force development in the leg press was 28767±8570 N/s (pre1); 25119±8945 N/s (pre2); 16684±7404 N/s (middle, $p<0.001$); 16720±7362 N/s (post $p<0.001$). Significances compared to pre2 measurement. The changes in physical performance did not relate with the energy balance. Plasma serum cortisol (from 498 to 648nmol/L) and SHBG (from 37.1 to 41.3nmol/L) concentrations increased significantly ($p<0.001$) and serum total testosterone (from 21.1 to 14.9nmol/L) decreased significantly ($p<0.001$) in the middle measurements compared to pre 2 measurements. In the post measurements the cortisol was normalized on the pre2 levels. Testosterone regained slightly from middle to post measurement ($p<0.001$) but it still differed significantly from pre 2 measurement. The SHBG concentration increased more after the middle measurement, post and follow measurement levels differed significantly compared to middle measurement ($p<0.001$). In testo/cortisol-ratio there was a significant decrement in the middle measurement compared to pre 2 measurement ($p<0.001$). Testo/SHBG-ratio decreased significantly in middle and post measurements compared to pre 2 measurement (both $p<0.001$). IN CONCLUSION: High physical loading and sleep deprivation or lack of rest was enough strenuous to affect the physical performance during the field exercise. Maintenance in muscular strength may be facilitated by the energy balance and adequate sleep.

KEY WORDS: field exercise, physical performance, testosterone, cortisol, SHBG, body composition

CONTENTS

1 INTRODUCTION

Military training attempts to simulate the performance in wartime via operations and field exercises. Strenuous and prolonged physical activity must be maintained for days or even weeks, including periods of very high energy expenditure which often involves carriage of heavy weights of equipment. Soldiers are often exposed to many other streuussors, such as sleep deprivation, energy and fluid deficiency, severe weather conditions, extreme ambient temperature and time pressure. Areas concerning in physiology are physical performance, endurance, muscle strength, thermoregulation and ability to concentrate. (Opstad, 1995, McCaig et al. 1986).

Several studies have presented prolonged physical work without adequate rest and sleep. Physiological consequences of the soldiers in different circumstances has been studied all over the world during many decades. Field exercises normally last a couple of days, one week or even longer (Nindl et al. 2002, Opstad 1995, Opstad et al. 1994, Rognum et al. 1986, Symons et al. 1988, Young et al. 1998). During prolonged military operations cardiovascular and respiratory capacities, neuromuscular performance and inadequate energy may be the limiting factors in physical performance (Opstad, 1995). In the real wartime situation lack of sleep is reality and it has been simulated in many military studies to investigate its physiological effects. Sleep deprivation might have an effect on hormonal balance and or physical performance (Opstad et al 1983, Haslam 1984, Martin et al. 1986, Legg et al.1987, Gomez-Medino et al. 2002, Abedelmalek 2013). Energy balance might be difficult to maintain because of high energy expenditure and lack of time to eat properly or difficulties to carry enough food.

Food is vital to keep the moral of the troop in field combat exercises as well as contributing to health and performance (Friedl & Hoyt 1997). Many kinds of rations are used during field exercises attempting to correspond increased energy expenditure, although nutritional requirements are hard to fill. (McCaig et al. 1986.) Operational rations must be easy to prepare and taste good, which helps eating both in training and in combat. (Friedl & Hoyt 1997.)

There are many studies that have investigated thermoregulation, physical performance or energy balance in cold environment (Castellani et al. 2003, Castellani et al. 2006).

Measured energy expenditures have been about 4500 kcal per day or even more during field exercises in the winter conditions from 3.5d to a couple of weeks (Hoyt et al. 1991, Castellani et al. 2003). In the summer during marches soldiers have to carry heavy loads of ammunition long distances on foot. In the winter most cross-country movement is on skis or snowshoes with loads of 30-35kg on the back, while heavy loads are moved on sledges. (McCaig et al. 1986.) Burstein et al. (1996) concluded that energy expenditure is primarily determined by the level of activity rather than by the climate conditions. They assessed the energetic status of soldiers exposed to intense physical activities in summer and winter condition. Results showed energy expenditure of 4281±170 kcal/day for the winter and 3937±159 kcal/day for the summer groups. Energy intake was almost identical between two groups (in the winter group 2792±124 kcal/day) and a negative energy balance of 1422±163 kcal/day and 924±232 kcal/day occurred in the winter and summer groups, respectively. The difference between the groups was not statistically significant. (Burstein et al. 1996.)

Castellani et al. (2006) and Hoyt et al. (2006) measured energy expenditure and energy balance between men and women in the winter and summer conditions, respectively. Both resulted that men had higher energy expenditure than women (25.7-26.6 MJ/d and 19.8-21.9 MJ/d, respectively) but there were no differences between men and women in the total energy expenditure normalized to body mass. In the summer condition study, weight-specific total energy expenditure was 343±26 kJ/kg/d and 354±18 kJ/kg/d between men and women, respectively (Hoyt et al. 2006). In the winter conditions, total energy expenditure normalized to body mass was 0.35±0.05 MJ/kg/d for men and 0.34±0.06 MJ/kg/d for women (Castellani et al. 2006). In both studies physical activity level (PAL) was almost the same between the sexes and also between the studies. In Castellani's et al. study (2006), men's PAL was 3.4±0.5 and women's 3.3±0.4 and in Hoyt's et al. study (2006), PAL was 3.5±0.2 and 3.6±0.3 for men and women, respectively. Physical activity level was calculated by dividing total energy expenditure by the calculated basal metabolic rate (Castellani et al. 2006).

The purpose of the study is to examine physiological consequences on 8-day strenuous military field exercise. We study the possible changes in physical performance - the maximal voluntary isometric contraction and rate of force development on the upper and lower extremities. In addition, the energy balance and changes in body composition; and the anabolic and catabolic hormonal (serum testosterone, cortisol and carrier protein SHBG concentrations) responses to the 8-day field exercise. We also observed if there is a connection between changes in body composition or maximal strength and energy balance during the field exercise.

2 EFFECTS OF PHYSICAL LOADING ON VARIOUS VARIABLES

Heart rate (HR) is traditionally used to monitor endurance-type training intensity during exercise. However, there appears to be a small day-to-day variability in HR. (Achten et al. 2003, Morton et al. 1990.) There are many other reliable ways to quantify training load via subjective or objective methods for example wrist-worn activity monitors or foot pedometer (Brugniaux et al. 2008).

Physical loading is versatile on military field exercises because of long workdays, abundance of ambulatory activities, carrying heavy loads and moving long distances by foot or skies (Burstein et al. 1996, Castellani et al. 2006, Hoyt et al. 2006, Nindl et al. 2002).

2.1 Effects of physical loading on energy expenditure

The higher physical activity, the higher energy expenditure. Soldiers have high physical loading on military field operations which increases energy expenditure. It can be estimated via many methods, for example by the heart rate method or sensor accelerometer activity meters (Brugniaux et al. 2008), double labelled water method (Schoeller et al. 1986) or intake-balance method where with the information of energy intake and changes in body composition (Hoyt et al. 1991). Metabolic cost can also be estimated from oxygen consumption or by direct calorimetry (Lyons et al. 2005) but these methods require collecting expired air during the measurement period and, therefore, are impractical for determining the energy requirements of military personnel in operational environments.

In the prior studies total energy expenditure has been from 3- to 5-fold according to different weather conditions. (Rognum et al. 1986.) Hoyt et al. (1991) resulted of energy expenditure of US Marines training in mountainous, cold-weather conditions also as 3-4-fold the resting metabolic rate. Hot and cold temperatures affect also energy expenditure (Burstein et al. 1996) like individual's basal metabolic rate, age and sex (Hoyt et al. 2006 & Castellani et al. 2006).

Lyons et al. (2005) performed an interesting protocol to assess the physiological consequences while participants walked on a treadmill at 4 kph (1.11 m/s) for 60 min on gradients of 0, 3, 6 and 9% whilst carrying backpack loads of 0, 20 and 40 kg. During the final 3 min of each 5-min exercise bout, indirect respiratory calorimetry and heart rate data were collected and the 'steady-state' metabolic VO_2 and cardiovascular (heart rate) demands quantified. Heart rate and oxygen uptake were both greater on every gradient when carring 20- or 40-kg load compared to 0-kg load. The energy expenditure per kg of load carried is equal to the energy expenditure per kg of body weight up to approximately 30 kg. The metabolic demands (% VO_{2max}) of the 0- and 20-kg load-carriage tasks were closely associated with relative VO_{2max} (ml/kg/min). Carrying increasingly heavier loads, humans become less efficient and the energy expenditure per kg of load increases sharply. The Lyons' et al. (2005) data are consistent with this view. This may explain why, during their study, the correlation between the metabolic demand (%VO_{2max}) of exercise and absolute VO_{2max} (ml/min) became progressively stronger as the load increased. As the load increases and subjects become less efficient, a high absolute VO_2 (ml/min) reserve is essential for heavy load-carriage tasks. (Lyons et al. 2005.)

2.2 Effects of physical loading on body composition

Many studies examining military endurance training courses include sleep deprivation and reduced-calorie diet. In Väänänen et al (2001) study soldiers' physiological and psychological loading during a 4-day march was investigated without the restriction of amount on sleep or energy intake. The 4-day march study showed an average weight reduction after each march. Daily mean decrease in scale weight was 1.6kg which indicated slight dehydration. Reduction was compensated for by the next morning. In this study, energy intake was not restricted. (Väänänen et al. 2001) In this study acute changes in body mass was due to decrease in total body water and depletion in muscle glycogen.

Castellani et al. (2003) concluded that even a short-term, 3.5 d (eighty-four hours), sustained military operation decreased body weight 3.9kg, % body fat 1.6% and fat free mass by 1.8kg. Also Nindl et al. (2002) reported a rapid weight loss during 72-hour military operation. Body mass declined 2.5kg, total soft tissue lean and fat mass declined ($P \leq 0.05$) by 1.5kg and 1.2kg respectively. Percent body fat decreased from 18.5±5% to 17.7±6%. A longer, 7 d, field exercise was arranged in Hoyt’s et al. study (2006) where male cadets’ body fat reserves decreased 45±15% and female cadets used 37±10% of their fat reserves. These results were similar to Rognum et al. (1986) where the fat reserve depletion was 50% in male cadets during almost the same kind of field exercise training. We can assume that 5% body fat is a minimum in men and 10% in healthy young women (Hoyt et al. 2006). In these studies, training load consisted of normal military activities and tasks. The endurance type loading took almost the whole day including simultaneous strength performances, and there was only 0-4-hours total rest or sleep.

In the study of Schiotz et al. (1998), 22 trained college-age male Reserve Officers Training Corps cadets participated in two different training program, periodized and constant-intensity strength training for the 10-week mesocycle. In both groups supervised training included resistance training exercise session plus aerobic running 4 days a week. Both groups consumed their normal diet, and they avoided all supplemental exercise during the training period. After the 10-week training period, the both groups showed changes in anthropometric measures. There were decrease in body fat percentage and increase in lean body mass. The periodized training groups had a significant decrease in % fat, from 11.6% to 9.9% and constant-intensity group non-significant from 10.4% to 9.7%. In lean body mass periodized group non-significant 1.2% increase and constant-intensity group 0.6% increase. Body mass showed non-significant change. Within groups relative training volume was not significantly different. (Schiotz et al. 1998.)

It is interesting that dehydration does not always occur when total body water is significantly decreased after prolonged military exercise. O’Brien et al. (1996) measured total body water (TBW) with D_2O before and after an 8-day, moderately cold-weather (1–3°C) military training exercise. They observed a significant decrease in TBW over the study, which they believed was due to a noticeable decrease in lean body mass and fat mass. They concluded that body fluid balance was maintained during this experiment despite high activity levels, significant body mass loss and negative energy balance. Significant dehydration did not occur as assessed by blood and urinary parameters. (O’Brien et al. 1996.)

2.3 Effects of physical loading on hormones

Exercise is known to be a powerful stimulus for the endocrine system. The hormonal response to exercise is dependent on several factors including the intensity, duration, mode of exercise (endurance versus resistance), and training status of the subject. (Karkoulias et al. 2008.) Moderate physical exercise does not cause any changes in the plasma levels of the catabolic hormone cortisol and the anabolic hormone testosterone compared with the concentrations during a control period. Intense physical exercise leads to an increase in plasma cortisol and a decrease in plasma testosterone compared with controls. Prolonged exhaustive physical exercise in men results in a decrease in plasma testosterone even down to normal female levels, and there is a constant increase in SHBG resulting in very low free testosterone concentrations. The concentrations of several hormones are known to affect muscle protein synthesis for example testosterone, human growth hormone and cortisol. (Adlerceutz et al. 1986.)

Cortisol, a glucocorticoid, is a catabolic hormone. Glucocorticoids are secreted by the adrenal cortex and released via the hypothalamic-pituitary-adrenal axis in response to physical or psychological stress and have profound effects on almost all physiological functions. Cortisol stimulates energy mobilization at many levels; first of all cortisol stimulates gluconeogenesis. (Opstad 1995.) But the catabolic effects of cortisol result in a decrease in protein synthesis and an increase in rates of protein degradation. (Kraemer et al. 1995.) Cortisol largely binds to plasma proteins, especially to the cortisol-binding globulin (CBG) when released into the blood stream. (Opstad 1995.)

Testosterone is an anabolic hormone and it works by increasing rates of protein synthesis and inhibiting protein degradation. It is one of the most potent naturally secreted androgenicanabolic hormones. (Vingren et al. 2010.) Most testosterone is synthesized and secreted by the Leydig cells of the testes via the hypothalamic-pituitary-gonadal axis. Small amounts of testosterone are also derived from the ovaries, adrenals and via conversion of other androgens. (Crewther et al. 2006.) Androstenedione and dihydroepiandrosterone are also secreted from the testis but at a rather low rate. Circulating androgens are predominantly bound to the transport protein sex hormone-binding globulin (SHBG) A change in SHBG concentrations may influence the binding capacity of testosterone and the magnitude of free testosterone available for diffusion across the cell membrane to interact with membrane-bound steroid receptors. Like cortisol, testosterone also exhibits a circadian pattern, with early morning peaks and the lowest value in the evening. (Opstad et al. 1995.)

Testosterone is important for the desired adaptations to resistance exercise and training; in fact, testosterone is considered the major promoter of muscle growth and subsequent increase in muscle strength in response to resistance training in men. In general, testosterone concentration is elevated directly following heavy resistance exercise in men. Age also significantly affects circulating testosterone concentrations. Children do not experience an acute increase in testosterone from a bout of resistance exercise; after puberty some acute increases in testosterone from resistance exercise can be found in boys but not in girls. Aging beyond 35–40 years is associated with a 1–3% decline per year in circulating testosterone concentration in men and similarly, aging results in a reduced acute testosterone response to resistance exercise in men. (Vingren et al. 2010) Human growth hormone is also an anabolic hormone which is secreted by the anterior pituitary gland and many of its anabolic effects are mediated through insulin-like growth factor 1 (IGF-1). Like testosterone, growth hormone works by increasing protein synthesis rates and inhibiting protein catabolism. (Crewther et al.2006.)

With respect to exercise, anabolic hormone testosterone is especially important in the growth and maintenance of skeletal muscle, bone and red blood cells. Like cortisol, testosterone also increases linearly response to exercise once a specific intensity threshold is reached. But however, even low intensity but prolonged exercise can result in significant elevations the testosterone and the same for cortisol. (Väänänen, 2002.) Guglielmini et al. (1984) suggest that serum testosterone increases during physical activities lasting up to three hours and decreases to or even below the pre-exercise values for longer physical efforts. They determined serum testosterone concentrations before and after physical activities of different duration (competitive walkers, middle distance runners, marathon runners and ultramarathon runners). (Guglielmini et al. 1984.)

According to Villanueva et al. (1986) and Lunger et al. (1987), endurance training implies hypercortisolism. Some deleterious effects of prolonged hypercortisolism are impaired microbial killing capacity, muscle catabolism, depression and anxiety. Exercise minimum 60% of VO_{2max} may cause an increase in the secretion of cortisol. Cortisol’s release, for example, affects metabolism by attempting to help maintain blood glucose levels during the exercise. (Brownlee, 2005.) But in endurance-trained men, 24 h cortisol secretion under nonexercising conditions is normal. (Duclos et al. 2007.) Walker et al. (1997) resulted the circannual rhythmicity of cortisol excretion in endurance-trained male. The highest concentrations of morning plasma cortisol were evidenced during winter and fall compared with spring and summer. The investigated circadian rhythms were almost normalized after 4-5 days of rest (Opstad 1994).

Endurance type overtraining decreases serum testosterone (Fry et al. 1998). Similarly Lucia et al. (2001) showed that during a three week´s extreme physical stress induced by cycling contest results in a significant decrease in serum testosterone concentration without a significant change in body weight or gonadotrophin levels. The testosterone response varies according to length of training session and amount of volume or intensity of exercise. It increases, decreases or remains unaffected following an acute resistance training or endurance type training bout. Karkoulias et al. (2008) determined the steroid hormonal responses to the marathon in a group of well-trained, middle aged, non-elite athletes. They took blood samples one week before, immediately after the race and one week later. Serum cortisol increased significantly after the race and returned to baseline one week later, serum testosterone decreased significantly and returned similarly to baseline one week after the race. (Karkoulias et al. 2008.) Ishigaki et al. (2005) arranged an 8-day strenuous training camp where the participants ran 284.1±48.2km during the camp. Serum cortisol concentration increased significantly ($p<0.001$) and serum testosterone concentration decreased significantly ($p<0.01$), and the ratio of testosterone to cortisol dropped by 50% after training ($p<0.001$). (Ishigaki et al. 2005.)

In the study of Brownlee et al. (2005), participants gave blood samples at rest and after exercise, at 1 hour into recovery from intensive exercise. Thee modes of exercise were running, cycling, rowing all of an intensity 65-75% VO_{2max} and exercise duration 60-90minutes. Cortisol, total testosterone and free testosterone were all analysed. As a result the Exercise Recovery samples for total testosterone and cortisol correlation were significant (TT vs. C, $r=-0.53$; $p<0.05$) but the resting samples were not significant ($p>0.05$) including free or total testosterone and cortisol. But interestingly, a significant positive relationship developed between cortisol and free testosterone following exercise (fT vs C, $r=+0.21$; $p<0.05$). This finding could support the notion that the observed testosterone reduction following certain forms of physical exercise could be related to cortisol elevations in response to that exercise. Since testosterone and cortisol are formed from the same precursor (in the same cascade of the reactions of adrenal gland), they remind structurally similar. Testosterone is transported to the circulation by the SHBG and other carrier proteins; and cortisol transported by the latter and cortisol binding globulin. These two hormones might compete for binding sites of the same carrier proteins. Thus the possibility on this exist an increased concentration of cortisol in circulation could cause some dissociation of free testosterone. (Brownlee, 2005.) Exercise appears to allow for the development of negative relationship between cortisol and total testosterone. But interestingly free testosterone has an opposite relationship with cortisol to that free total testosterone.

Some studies have reported negative relationships between circulating testosterone and certain stress hormones (for example cortisol) in humans. That may be the reason why the subclinical resting testosterone levels are often found in some endurance-trained males. These relationships may also relate to exercise. Daly et al. (2005) examined the relationship between total and free testosterone levels and cortisol following prolonged endurance exercise in trained males. Twenty-two endurance-trained males volunteered to run at 100% of their ventilatory threshold (VT) on a treadmill until volitional fatigue. Blood samples were taken at pre-exercise baseline (B0); volitional fatigue (F0); 30 min (F30), 60 min (F60), and 90 min (F90) into recovery; and at 24 h post-baseline (P24 h). Exercise induced significant changes found ($P<0.05$) from B0 to F0 in total testosterone, cortisol. Both of these hormones were still significantly elevated at F30; but at F60 only cortisol was greater than their respective B0 values. Free testosterone displayed no significant changes from B0 at F0, F30, or the F60 time point. At 90 min into recovery cortisol was not significantly different from baseline values, but total testosterone and free testosterone were reduced significantly from baseline. Cortisol, total testosterone and free testosterone at 24 h post baseline were significantly lower than their respective baseline levels. Negative relationships existed between peak cortisol response (at time 30min recovery) versus total testosterone (at 90min recovery, $r=-0.53$, $P<0.05$; and at 24 h post baseline, $r=-0.60$, $P<0.01$). In conclusion, the present findings give credence to the hypothesis suggesting a linkage between the low resting testosterone found in endurance-trained runners and stress hormones, with respect to cortisol. (Daly et al.2005.)

Testosterone/cortisol-ratio alters either testosterone or cortisol concentration changes. This reflects well anabolic-catabolic stage. Remes et al. (1985) resulted that the 21km march elicited a significant increase (by 14%, $P<0.02$) in the mean plasma SHBG level. Concomitantly with the mean plasma testosterone decreases, the mean T/SHBG-ratio also decreased during both control and exercise period.

Sgrò et al. (2014) studied acute adaptive role of the hypothalamic-pituitary-testicular (HPT) axis to standardized sub-maximal endurance exercise in a laboratory setting. They investigated the correlations between testosterone and classic adaptive hormones variations. Twelve healthy male volunteers cycled a 30-min sub-maximal exercise on cycle ergometer at individual anaerobic threshold and a maximal exercise until exhaustion. Serum, cortisol, testosterone (total (TT), calculated free (cFT) and bioavailable (cBioT)), SHBG, and other variables and their respective ratios were evaluated before and after exercises. Blood samples were collected 30min, 15min and immediately before, immediately after and at different time points during recovery

(+15, +30 and +60 min) for hormones assays. Oxygen consumption and lactate concentration were evaluated. As a result testosterone (TT, cFT and cBioT) acutely increased in all volunteers after both exercises. Testosterone increased in parallel to GH after both exercises and to cortisol only after maximal exercise. Differently from other increased hormones, testosterone increases were not correlated to exercise-intensity-related variables. The anabolic/catabolic steroids ratios were higher after sub-maximal exercise, compared to maximal. Sgrò et al. (2014) concluded that a 30-min sub-maximal endurance exercise acutely increased serum testosterone similarly to maximal exercise, but without cortisol increases. Exercise-related testosterone peaks should be considered adaptive phenomena, but few data on their short- and long-term effects exist. Investigations on the mechanisms of adaptation to exercise in active individuals with physiological or pathological hypo-testosteronemia are warranted. Similarly Meckel et al. (2009) resulted that maximal exercise (4x250-m run at 80% of maximal speed) was associated with significant increase in testosterone, GH and testo/cortisol ratio but insignificant effect on cortisol levels. In both studies testo/cortisol ratio after sprint interval exercise suggest exercise-related anabolic adaptation. (Meckel et al. 2009.)

Otherwise an acute heavy resistance exercise did not affect testosterone concentration in previously fit athletes in a study by Kraemer et al. (2001). In Karila's et al. (2008) study participants did anaerobic resistance type training leading reduced testosterone serum concentration but the training was combined with caloric deprivation and weight loss. Blumert et al. (2007) arranged the protocol where they investigated the weightlifting performance after 24 hours of sleep loss. Testosterone and cortisol levels were quantified before, immediately after and 1 hour after the resistance training event. Compared to pre-exercise levels in the nonsleep deprived conditions cortisol concentration levels decreased immediately after the exercise and 1-hour post exercise. Testosterone concentration remained unaltered. Exercise was high intensity, moderate volume. Also diural variations could have been obscured the possible increase.

Hormonal parameters have been studied in many military studies. Gomez-Merino et al. (2003) studied immune and hormonal changes following military training (three week combat training followed by 5-day military course). As a result significantly lowered testosterone (from 15.1±0.7 nmol/L to 9.8±0.6 nmol/L) reflects a general decrease in steroid synthesis as a consequence of the physical and psychological strain. Changes in body mass index, or mean plasma cortisol were not significant.

The purpose of Väänänen et al. (1997, 2002, 2004, 2005) research series was to describe the physiological responses to daily repeated acute prolonged exercise during a 4-day march (the "International Four-Day Long-Distance March" in Nijmegen, The Netherlands in 1993 and 1994) and a 2-day cross-country ski event (a 2-day Finlandia Ski Race in Lahti, Finland in 1995). Physically active army officers participated in a 4-day march totaling 165-185 km while carrying 10-kg backpacks, (in 1993 n=6 and in 1994 n=15). Daily walking time was 7-10 hours and average heart rate during walking was 59% of maximum heart rate. In the skiing study there were, as a subject, ten physically active men who skied 50 km per day on both days, duration of 3 hours, and average heart rate during skiing was 87% of maximum heart rate.

The purposes of the protocols were to investigate the resting levels and the acute hormonal responses of serum testosterone and cortisol and other variations also. In the skiing study, venous blood samples were obtained before and after skiing, and after 1 week's recovery, to determine the concentrations of testosterone and cortisol in the blood. Testosterone was reduced by over 20% after both days (p=0.016 and 0.002, respectively) and cortisol increased 2.2- and 2.6-fold after the races (p<0.001). (Väänänen et al. 2004.) The marching protocols resulted in the adrenal cortex the increased response to acute exercise, could be seen after the 1st day. The acute increasing effect of a single walking session on cortisol was seen only after the first day when there was a 60% increase. The concentration of testosterone was reduced after the first two exercise sessions (when they were decreased by 18-22%) and a plateau was reached after the 3rd day of walking. The acute response of the adrenal cortex and pituitary-gonadal axis disappeared within 4 days of repeated prolonged walking and no dramatic lasting changes occurred despite this major 4-day effort. (Väänänen et al. 1997 & 2002.) Responses in cortisol concentrations after skiing (where cardiorespiratory loading was higher) were greater than after marching and responses in testosterone was similar in both studies.

The protocols demonstrates that the pituitary-gonadal axis and the adrenal cortex adapted to four days of repeated moderate 8 h walking, but not so well to two days of repeated strenuous 3 h skiing. Heavy physical stress alters the acute responses of both the adrenal cortex and the hypothalamus-pituitary-testicular axis. (Väänänen et al. 2004.)

In older studies, during the ranger training course there is a 90% decrease in the plasma levels of both free and total testosterone and dihydrotestosterone. The decrease in testicular androgens was mainly due to the physical strain since no significant effect was found when cadets were given extra food. (Opstad & Aakvaag, 1983.)

In the studies where the effects of sleep deprivation and or physical activity on hormonal responses are investigated the decrease or increase in serum hormone concentration can be explained also by diurnal variations which could have masked the possible increase or decrease. (Häkkinen et al. 1988)

2.4 Effects of physical loading on physical performance

Physical activity and training improve the long term results in strength and physical performance (Schiotz et al 1998) but in the short-term muscle strength and physical performance might hinder. Schiotz et al. (1998) also concluded with the support of previous studies that increase in muscular strength in long term is directly related to increases in lean body mass.

The Väänänen’s et al. (2002, 2004, 2005) research series measured also the functional strength, flexibility, and ranges of motion of the lower extremities in all protocols. And leg measurements showed no signs of edema, decreases in flexibility, or functional strength. As a conclusion subjects with excellent aerobic fitness are able to ski at high intensity a total of 100 km over 2 successive days or walk at moderate intensity a total of 185 km over 4 days without any major adverse effects on the musculoskeletal system. These studies indicated that daily repeated long lasting acute but non-competitive walk and skiing of intensity at approximately 60-90% of the maximum heart rate is well within the physiological capabilities of soldiers who are in good physical condition with good aerobic capacity.

The physical effects of ultra-long exercise were monitored in fifteen Finnish soldiers participating in an International Four-Day Long-Distance March under the field conditions in the Netherlands. The march was 165km long, corresponding to a total of 200 000-250 000 steps. Participants were healthy, physically active but non-athletic army officers and cadets. The subjects carried a 10-kg backpack, wore army uniforms and combat boots. The subjects had water and snacks freely available. The functional muscle strength of the lower limb was measured by performing three maximal static jumps and three maximal countermovement jumps on the day before the first march and within 1 hour after the last march. The marching period did not affect the results gained from maximal voluntary vertical jumps, suggesting that the neuromuscular performance of the lower legs remained unchanged. Roughly estimated the energy consumption during the marches was ~390 kcal per hour and the mean heart rate level ~60% of the maximal rate. Energy is produced aerobically at this consumption level, especially through the breakdown of fatty acids. There was no energy deficit so soldiers were easily able to maintain their performance capacity. (Väänänen et al. 2001.) Similar results were presented in earlier study from Väänänen et al. (1997) in which a smaller group of middle-aged men walked through the same 4-day march.

Schiotz et al. (1998) resulted that the dynamic 1-RM strength for the bench press and parallel squat increased; both groups improved 1-RM bench press strength pre- to post-testing, periodized significant 8.3% and constant-intensity non-significant 5.0%. Parallel squat 1-RM strength improved significantly, in periodized 9.7% and constant-intensity 11.2%. Both groups also showed an improvement in other physical performance tests (sit-ups, push-ups, 2-mile run and ruck-run). In this study 22 trained college-age male Reserve Officers Training Corps cadets participated in two different training program, periodized and constant-intensity strength training for the 10-week mesocycle. In both groups supervised training included resistance training exercise session plus aerobic running 4 days a week. Both groups consumed their normal diet and they avoided all supplemental exercise during the training period. (Schiotz et al. 1998.)

Rissanen et al. (2005) have concluded that maximal force do not change during prolonged 12-day military operation. Rissanen et al. (2005) also showed an interesting result that even if maximal force of leg extensors did not change but the rate of force development production decreased from the initial value. Maybe the duration of the military operation has been insufficient and had a minor effect on soldiers' maximal neuromuscular performance. (Rissanen et al. 2005.) Moreover, the body mass loss may have not been enough to decrease physical performance (Fogelholm et al. 1993).

In these studies even the acute physical performance has not been enough hard to impair neuromuscular capacity significantly. Body composition changes enough either. There have been more studies where participants have influenced under heavy physical activity but not enough energy deficit.

3 NEGATIVE ENERGY BALANCE AFFECTS VARIOUS VARIABLES

Energy expenditure of the physical activity is only a partial amount of the total daily energy expenditure. EE from physical activity is only 25% of TDEE with the person who is inactive while endurance athlete's EE from physical activity can be 50% of TDEE on training days. Individual working hard labour can have the same percentages. (Bouchard, Blair & Haskell 2007, 12.)

Energy balance is the commonly assessed variable in many studies interested in military operations (Hoyt et al. 2006, Nindl et al. 2002). Total energy expenditure (TEE) can be measured, for example, using double labelled water technique and daily energy intake is assessed from detailed food records. Energy balance is calculated as a difference between energy expenditure and energy intake.

A wide range of studies have resulted an insufficient energy intake compared with the high energy requirements. (Burstein et al. 1996, Hoyt et al. 2006, Nindl et al. 2002.) Hoyt et al. (1991) measured adult male Marines during a strenuous 11-day cold-weather field exercise. The subjects kept food diaries of rations consumption. Hoyt et al.

resulted daily energy intake as 3,132±165 kcal/day. Rognum et al. (1986) reported an average food energy intake of 33 MJ/d (~8000 kcal per day) and it was needed to maintain body weight. In Castellani's et al. (2003) study participants went through the short-term 3.5d sustained military operation and energy intake was approximately 1650 kcal/d. Hoyt et al. (2006) wanted to build up the negative energy balance conditions where participants were allowed to eat only 0.2-1.9 MJ/d (~50-460 kcal per day) which is 1-9% of the cadet's energy needs.

There are several kind of rations; McCaig et al. (1986) presented energy supply which was predominantly by packs containing food for a twenty-four-hour period. The General Service (GS) Pack based on tinned foods has an energy value of about 16,83MJ (~4085kcal), whereas the Arctic Pack uses dehydrated foods and has an energy value of 18,95MJ (~4599kcal) to allow for the increased energy requirements in cold weather. As a result of questionnaire the Arctic ration pack was favoured because the packets of dehydrated food could easily be carried in pockets. DeLany et al. (1989) used ready-to-eat meal diet and a 2000 kcal/day lightweight rations.

McCaig (1986) denotes Wyant et al. (1983) study that arduous activity in the cold predisposes to dehydration both because of excess losses as sweat or from the respiratory tract, and because of reduced intake due to voluntary dehydration or the difficulty of obtaining water. Sometimes water supply is hindered because the lack of running water or snow. Water is used each day in the preparation of food and drinks. In the winter conditions water is often intended to come from melted snow. Though, the melting of snow is a time-consuming process and a disincentive in maintaining adequate hydration. (McCaig 1986.)

3.1 Effects of negative energy balance on body composition

In the Castellani et al. (2006) study the participants were under negative energy balance and sleep deprivation during 54 hours. Thirty men U.S. Marine recruits showed total energy expenditure 25.7 MJ/day, normalized to body mass 0.35±0.05 MJ/day/kg. TEE was measured using doubly labelled water ($D_2{}^{18}O$). Energy intake was 6.0±2.0 MJ/day which was estimated using beverage diaries and collecting ration wrappers saved by each volunteer. Carbohydrates 50±6%, fats 37±6%, proteins 14±3%. Total mean energy deficit over the FEX was -43±10.4 MJ. The clear energy deficit conducted the body mass loss -3.1±0.8kg during 54 hours. Study did not reveal the actual fat free mass and fat mass changes but the researchers used these results by calculating energy expenditures normalized to body mass and corrected fat free mass. (Castellani et al. 2006.)

The purpose of Rognum's et al. study (1986) was to investigate if the performance of subjects exposed to a period of sustained, strenuous exercise and sleep deprivation could be maintained by reducing the energy deficit to a minimum by feeding a carbohydrate rich diet. Rognum et al. (1986) reported an average food energy intake of 33 MJ/d, and it was a minimum needed to maintain body weight. In this study, they compared high- and low-energy diets on healthy male cadets during prolonged heavy exercise. Other group received a diet providing 33.49MJ (over 8000kcal) and other providing 6.30MJ (1500kcal). Both groups received a basic diet containing about 125g of carbohydrates, 70g fat and 100g of protein with an estimated daily energy content of 1500kcal. The high-energy group received an additional 6400kcal per person per day, consisting of 1230g carbohydrates, 120g fat and 105g protein, and a total of 4 to 5 litres of liquid. The latter groups used nearly tasteless and colourless maltodextrin to achieve the high carbohydrate content. Both groups were allowed to drink when needed. Participants in the low-energy group drank daily about 4 to 5 litres of water but men in high-energy group had no desire to drink extra liquid. During the measurement period for 107 hours, participants engaged in almost continuous simulated combat activities with less than two hours of sleep during the entire period. The mean loss of body weight was about six times greater in the low-energy group (LE) than in the high-energy group (HE). The average loss of body-fat in the high-energy group was 1.3kg compared with 3.1kg in the low-energy group, suggesting that even a high-energy group had energy deficit. The HE group lost about 10% of their body-fat and, while LE group lost about 24%, the differences being statistically significant. They did not assess the daily energy expenditure but comparing body composition changes and energy intake conclusions can be made about energy balance. (Rognum et al. 1986.)

Huovinen et al. (2015) investigated the effects of a 4-week weight reduction on body composition, physical performance and serum hormones. Two groups of male track and field jumpers and sprinters had energy restriction and high protein and reduced carbohydrate intake. High weight reduction group (HWR, n=8) and low weight reduction group (LWR, n=7) had energy restriction 750 kcal/d and 300 kcal/day respectively. As a result total body mass and fat mass decreased only in HWR group by 2.2±1.0kg and 1.7±1.6kg respectively. Fat free mass did not change significantly. Similarly Zachwieja's et al. (2001) controlled clinical research resulted total body mass loss of 1.29±0.16kg ($p<0.001$) after the 24-day dietary energy restriction (750kcal/d). The lean body mass declined for 61% of the weight loss. (Zachwieja et al. 2001.)

3.2 Effects of negative energy balance on hormones

In the versatile physiological study of U.S. Army Ranger Training (Nindl et al. 2007) results show the changes for the somatotrophic hormonal biomarkers after 8-week. Testosterone was significantly lower, whereas serum cortisol was significantly higher (+32%) after U.S. Ranger Training; testosterone pre value was 17.3±4.8nmol/L and post 3.0±1.8nmol/L, cortisol pre 469±106nmol/L and post 692±109nmol/L. The post circulating testosterone value was very low comparing the lower limit of normal circulating testosterone value 10.4nmol/L. In this study the body mass decreased from 78.4±8.7 to 68.4±7.0kg, overall body mass decreased by 12.6%. Fat free mass decreased from 63.9±6.0 to 61.3±5.8kg, change -2.6±2.0kg, percentage change -4±3%. Fat mass decreased from 14.5±3.9 to 6.0±2.3kg, change -8.5±2.3kg, percentage change -141±19%. Body composition was measured by dual-energy X-ray absorptiometry. During eight week the daily energy deficit was 1000 kcal/day, except between the field instructions during different periods. Cortisol was correlated with losses of tissue mass but testosterone did not correlate. They concluded that weight loss (> 13% of body mass), IGF-I and cortisol correlate more closely with soft-tissue adaptations than does testosterone during severe week monitoring period. (Nindl et al. 2007.)

Guezennec et al. (1994) concluded that extra food might reduce the decrement of testosterone during a military training course. They compared different energy intakes and resulted that plasma testosterone concentration was decreased by 50% in the low energy diet (LC) in which participants received only 1800 kcal per day. The percentage of each class of nutrient was 47% carbohydrates, 35% lipids and 18% proteins. Testosterone concentration decreased by 20% in the medium energy diet group (MC) in which subjects got 3200 kcal per day (55% carbohydrates, 30% lipids and 15% proteins). In high energy diet group (HC) energy intake was 4200 kcal per day including energy percents of 60% carbohydrates, 25% lipids, 15% proteins. In HC group plasma testosterone concentration decreased 23% compared the values measured before the field exercise.

Karila et al. (2008) investigated the effects of a rapid weight reduction. The state was an authentic pre-competition condition and elite wrestlers as participants. After three weeks of weight reduction the mean weight loss was 8.2±2.3%, mean reduction of fat mass 16.2±6.9% and lean body mass 7.9±2.5%. In serum testosterone there was a significant decrement (63±33%, $p \leq 0001$) and in serum sex hormone binding globulin there was a significant increment (40±21%, $p \leq 0001$); significant dehydration was noticed. They found a significant correlation between reduced weight and decreased serum testosterone concentration. Karila et al. (2008) concluded that even a short-term weight reduction may have a marked effect on body composition, electrolyte homeostasis and hormonal parameters.

3.3 Effects of negative energy balance on physical performance

Fogelholm et al. (1993) concluded that a 5% loss of body mass gradually (3 weeks) or rapidly (5 days) has been shown to result in little or no decrements in physical performance.

Previous investigations have shown that during exercise at rates greater that 50% of maximal oxygen uptake, endurance is enhanced by carbohydrate-rich diets, whereas during exercise of lower intensity the composition of the diet is less important. Rognum et al. (1986) pointed out the results of Waldum et al. (1974) that soldiers during simulating combat situations exceed 50% of their maximal oxygen uptake for only short periods. In the study of Castellani et al. (2006), they found that despite the negative energy balance the participants still maintained high energy expenditure and physical activity levels (PAL 3.4±0.5). Many activities were strenuous and the recruits remained active for approximately 20 hours per day. (Castellani et al. 2006.)

Guezennec et al (1994) demonstrated how physical performance was lower in low-energy-intake-group (1800 kcal/day) than in the higher energy intake groups (3,200 and 4,200 kcal/day). During a 5-day military field exercise participants had 8,000-10,000 kcal daily energy expenditure, and they showed 15% decrease in cycling to exhaustion and 7% decrease in VO_2max. (Guezennec et al 1994). Nindl et al. (2007) sum up that short-term periods of energy imbalance have equivocal effects on physical performance but still there is not enough evidence of a more prolonged and dramatic energy deficit experienced in soldiers.

In the 8-week U.S. Army Ranger Training Course, the negative energy balance induced decrement in physical performance; maximal lifting strength using the incremental dynamic lift decreased from 81.5±13.3kg to 65.1±10.6kg, vertical jump height from 44.1±7.4kg to 39.9±6.2cm, and explosive power output from 3972±561 to 3119±479 W by 20, 16 and 21% respectively. Average daily energy deficit was about 1000 kcal/d. (Nindl et al. 2007.)

Nindl et al (2002) highlighted several studies which have resulted in 13-16% weight loss in body mass over a 61-d period. Those studies demonstrate that muscular strength and power output were degraded after severe weight loss. Studies involving a lesser and short-term energy deficits have shown minimal changes in physical performance (Fogelholm et al 1993). Friedl & Hoyt (1997) suggested in their review that body mass losses of 5-10% are necessary before any significant decrements in performance occur.

Huovinen et al. (2015) and Zachwieja et al. (2001) found the improvement or maintenance in weight-bearing power performance after 24-28 days of energy 750kcal daily energy restriction. Zachwieja et al. (2001) resulted also maintained 1RM muscle strength in leg and shoulder press.

Similarly Fogelholm et al. (1993) studied gradual and rapid weight loss effects on performance in male athletes. Participants were wrestlers and judo athletes and they were used to losing about 4-8% of body weight by a combination of sweating and excessive dietary and fluid restrictions before major competitions 5 to 15 times per year. Because of dehydration, rapid weight loss may impair aerobic, anaerobic and strength performance. A more gradual weight loss would probably maintain a hydrated state better. Present study consisted of two procedures, first participants lost weight over a three week by dietary restriction (gradual = GP) and during the second procedure (rapid = RP) the subjects were instructed to lose the same amount of weight as in the first procedure, but now in 2.4 days (59 hours). Physical performance measures were done before and after both weight loss procedures and tests included 1) sprint (3x30m with 4 to 5min rest), 2) vertical jumps in addition 0, 50 and 100% extra weight (3x3 jumps 30-60s interval with 3 to 5min rest between sets) and 3) anaerobic test (1-min Wingate test). Result showed that sprint performance was similar during the entire study. Vertical jump height with extra load (50% of weight) increased ($P<0.01$) after gradual weight loss. None of the jumping height results changed significantly during rapid weight loss. The unchanged or even improved jumping height results indicate preserved capability of the neuromuscular system to produce force. Interestingly rapid weight loss followed by 5-h loading did not impair speed, vertical jump or anaerobic performance. Fogelholm et al. (1993) list similar studies with opposite results: Houston et al. (1981) found a decreased strength and Horswill et al. (1990) and Webster et al. (1990) decreased anaerobic performance after rapid weight loss.

4 PHYSIOLOGICAL RESPONSES TO SLEEP DEPRIVATION

The vast majority, regardless of sex or race, prefer 7 to 8 hours of sleep per 24 hours. Sleeping during the day is less recuperative than during the night and continuous sleep is more effective than several short naps even if the total time of naps is more. Short naps, ten to 20 minutes, are useful when continuous sleep is not possible. During military operations adequate sleep is hard or even impossible to accomplish. Sleeping the preferred 7 to 8 hours per night the week before military field exercise may help prepare for optimal performance during military operation. (Giam 1997 & VanHelder 1989.) Several studies have presented prolonged physical work without adequate rest and sleep (Nindl et al. 2002, Opstad 1995, Opstad et al. 1994, Rognum et al. 1986, Symons et al. 1988, Young et al. 1998).

Researchers (Giam 1997 & VanHelder 1989) suggest that if there is an opportunity to obtain short periods of rest or sleep during the daytime, the effects of sleep deprivation are minimized also in those situations when the length of the night rest is shorter than the average reported length of nocturnal sleep during military service. Väänänen et al. (2001) conclusions were based on Partinen M. Sleeping habits and sleep disorders of Finnish men before, during and after military service article (1982).

4.1 Effects of sleep deprivation on physical performance

Sleep deprivation or partial sleep loss are common in work conditions as rotating shifts and prolonged work hours, in sustained military operations. Although it is well established that sleep loss has negative effects on mental performance, its effects on physical performance are equivocal." Vanhelder's (1989) review examines this question from recent studies published on this problem. Sleep deprivation of 30 to 72 hours does not affect the aerobic or anaerobic performance capability or muscle strength and electromechanical responses of individuals. Time to exhaustion, however, is decreased by sleep deprivation, because of an increase in insulin resistance and a decrease in glucose tolerance.

Martin et al. (1981) investigated the effect of sleep deprivation on tolerance of prolonged exercise. He arranged a heavy exercise performance (prolonged treadmill walking at 80% of the $VO2_{max}$ until exhaustion) after 36 hours without sleep and compared that after normal sleep in eight subjects. As a result sleep loss reduced work time by an average 11% ($p<0.05$) but half of the group showed only 5% and other half 15-40% decrement in exercise tolerance. Interestingly physiological variables remained unchanged (heart rate, metabolic rate, ventilation) which suggest that the psychological effects of an acute sleep loss may contribute to decreased tolerance of prolonged heavy exercise (Martin, 1981).

Blumert et al. (2007) arranged the protocol where they investigated the weightlifting performance after 24 hours of sleep loss. Testosterone and cortisol levels were quantified before, immediately after and 1 hour after the resistance training event. 1RM weight lifting performance did not alter after sleep deprivation.

Although it is well established that sleep loss has negative effects on mental performance, its effects on physical performance are ambiguous. Military personnel perform missions that include sleep deprivation as well as continual work and underfeeding (Bradley et al. 2002). Sleep deprivation of 30 to 72 hours does not affect cardiovascular and respiratory responses to exercise of varying intensity, or the aerobic and anaerobic performance capability. Muscle strength and electromechanical responses are also not affected. Time to exhaustion, however, is decreased by sleep deprivation. (VanHelder et al. 1989.) Symons et al. (1988) suggest that sleep loss of up to 60 hours will not impair the capability for physical work. They had 11 subjects who remained awake for 60 hours in the experimental condition. As a result of sleep deprivation for maximal isometric and isokinetic muscular strength and endurance of selected upper and lower body muscle groups were not significantly altered. (Symons et al. 1988.)

In opposite to previous results, Legg et al (1987) and Rognum et al. (1986) have suggested that sleep restriction affects physical performance and neuromuscular forces. Legg et al. (1987) monitored the physiological effects of a military 8-day artillery field exercise which was designed to include sustained manual handling of heavy artillery shells (45kg) and also partial sleep loss. The daily amount of sleep obtained by both groups (experimental and control) was similar (3 to 4 hours) as were their activity patterns and food and fluid intake. The experimental group prepared, handled and loaded artillery shells and charges, while the control group was instructed to simulate manual materials handling. The artillery soldiers as participants were only able to obtain naps accumulating to a daily average of 3.1 and 3.5 hours for the experimental and control groups respectively. The major finding in this study is a reduction in the upper body mean power of the experimental group but not of the controls. In other hand the isometric hand grip strength data suggest that sleep played a significant role since both experimental and control groups demonstrated a gradual progressive decrement in IRHGS during the trial, which became statistically significant (p<0.05) on day three. This may reflect a decrement in muscle fibre recruitment or an alteration in motor unit firing frequency. The decrease in mean power in only the experimental group suggests that there may also have been a partial depletion of upper body intramuscular energy sources. It is very interesting that IRHGS did not return to pre-trial values during the three post-trial recovery days. But it is still too forward to suggest that because the hand grip strength has been proven as an indicator of general muscular strength, it would be possible that general body muscular weakness may persist for at least three days following by a prolonged 8-day sustained high intensity military exercise. (Legg et al.1987.)

Rognum et al. (1986) studied a group of 24 men during a period of heavy, sustained work lasting for 107 hours, during which time they had less than two hours sleep. Nine men received a diet providing 33.49MJ (8000kcal) and 15 men a diet providing 6.30MJ (1500kcal) per day. The subjects were assessed by objective measurements of simulated military tasks. There were no statistically significant differences between the two groups of subjects in any measure of performance, but there was a significant decrement comparing from pre and post results. And also the high-energy group felt slightly more alert than did the low-energy group, the difference being statistically significant (p<0.05) on the day three. These results suggest that the major factor influencing performance and well-being in these experiments was sleep deprivation rather than the sustained physical activity or low energy intake. It is also concluded that the decline in performance could not be prevented by giving a high-energy diet alone. In conclusion we can emphasize how important even brief periods of sleeps are.

On the other hand, in the 1984 Haslam studied the performance of the soldiers after they deprived of sleep on sustained operations. Participants performed two 9 days tactical defensive exercises. On the first exercise three groups of infantry were scheduled either 0, 1.5 or 3 hours of sleep in every 24 hour following a period with no sleep at all. On the second exercise soldiers were scheduled for 4 hours on sleep in every 24 hours for a 6 days following 3.75 days without any scheduled sleep. Physical fitness, performance, and mood were assessed throughout both exercises. Haslam resulted that soldiers are likely to be military ineffective after 48-72 hours without sleep but after that a small amount of recovery sleep relative to the amount of lost has very beneficial effects. Results indicate that the effects of sleep loss are psychological rather than physiological. (Haslam 1984).

Kyröläinen et al. (2007) demonstrated that 60-hour sleep deprivation does not cause any dramatic changes in the short term neuromuscular functions. Participants, healthy male cadets (n=20, age 26±2 years, body height 1.77±0.01 m, and body mass 79.6±11.1.kg) were tested every evening and morning at 8:00 o'clock during 60 hours without no sleep at all. Tests included reaction tests, balance tests, goal-directed movement testing, eye-arm coordination, tendon tap reflex test and maximal isometric force production with EMG activities of the knee extensor muscles. Results showed that the maximal isometric force did not change (778±166 N vs.754±162 N) during the study. Neither any change appeared in the rate of force production and force-time curves compared between the 1st and 3rd evening results. Reaction time did not significantly change during the period, even if in the mornings reaction times were a bit longer than measured in the evenings. No statically significant changes were noticed in the other variables either. As a conclusion, people having good physical condition can maintain their vigilance and concentrate for the short term tests, at least during 60 h sleep deprivation. In the long term performances, some weaknesses in the neuromuscular performance may appear. Kyröläinen et al. (2007)

Vaara et al. (2009) concluded that sleep deprivation (60-hour sleep restriction) causes alterations in autonomic regulation of the heart and in thermoregulation. Heart rate variability was measured during an active orthostatic test in the mornings and evenings from twenty young healthy male cadets. Sleep loss resulted in increased vagal outflow, as evidenced by significantly decreased heart rate ($P<0.001$) while high frequency and low frequency power increased ($P<0.05$-0.001).

4.2 Effects of sleep deprivation on hormones

Disturbed sleep might have an effect on the nocturnal secretion of cortisol and GH and sleep-wake cycle. GH and cortisol secretion are closely related to the sleep-wake cycle;

GH is released during the first period of sleep while cortisol secretion is low but it increases dramatically during the early morning sleeping hours. These hormones not only depend on sleep but also often secreted in response to physical stress. (Abedelmalek, 2013)

In the acute effects Abedelmalek (2013) investigated the effects of partial sleep deprivation on plasma concentrations of testosterone and cortisol plus proinflammatory cytokine during a repeated sprint exercise. The arranged exercise was 4x 250-m treadmill run at 80% of individual's maximal speed at 8:00am after 4,5-hour (3:00am early awakening) and normal 8,5-h sleep duration. Blood samples were collected before, exactly after the first and fourth sprints and 60min after the exercise. They resulted that cortisol was not affected by the partial sleep loss, however testosterone was higher ($p<0.05$) 60min after the exercise following partial sleep deprivation.

Martin et al. (1986) deprived subjects of sleep prior to exercise (30min heavy and 3h light treadmill walking) to see if this altered the stress hormonal response to that exercise. They concluded that sleep loss did not alter the stress hormonal response (cortisol) during subsequent exercise.

Testosterone/cortisol-ratio alters either testosterone or cortisol concentration changes. This reflects well anabolic-catabolic stage. Remes et al. (1985) resulted that the 21km march elicited a significant increase (by 14%, $P<0.02$) in the mean plasma SHBG level. Concomitantly with the mean plasma testosterone decreases, the mean T/SHBG-ratio also decreased during both control and exercise period. The sleep deprivation did not cause significant changes in the mean plasma SHBG or T/SHBG ratio. (Remes et al 1985.) A slower decrease in testosterone happened when participants was given three hours of extra sleep each night. (Remes et al. 1985). The sleeping time represents the period when the hormonal profile is the mist anabolic, for example increased ratio of growth hormone to cortisol and of testosterone to cortisol (Kern et al. 1995).

Gomez-Medino et al. (2002) determined how 5-d military training course after 3-wk combat training affects serum leptine levels. They also analysed cortisol and testosterone from blood samples. Serum testosterone decreased significantly (<0.001), whereas cortisol was unchanged at the end of the course. Combination of heavy physical activity and sleep deprivation led to energy deficiency. But the training program had no significant effect on mean body mass index.

Opstad et al. (1983) investigated the effect of sleep on the serum levels of hormones during prolonged heavy physical strain and calorie deficiency. There were 19 young men participating in a 5-day ranger training course with a calorie consumption of 35 000-50 000 kJ per day (8 490-12 140 kcal per day), and a calorie intake of about 6 000 kJ per day (1450 kcal per day). The subjects were divided into two groups: the stress group included eight cadets and were allowed no organized sleep during the course, whereas the sleep group (nine cadets) had 3 h sleep each night. As results, small but significantly ($p<0.01$) higher serum levels were found in the sleep group compared to the stress group for cortisol, growth hormone and testosterone. The changes found during this investigation are similar to changes found during previous courses. All hormones were normal within 23 days after the end of the course.

5 PURPOSE OF THE STUDY

The purpose of the study is to examine physiological consequences on 8-day strenuous military field exercise.

5.1 Research objectives

1. To examine the effects of 8-day field exercise on physical performance - the maximal voluntary isometric contraction and rate of force development on the upper and lower extremities.
2. To examine the energy balance and changes in body composition during the 8-day field exercise.
3. To examine the anabolic and catabolic hormonal (serum testosterone, cortisol and carrier protein SHBG concentrations) responses to the 8-day field exercise.

4. To examine, if there is a connection between latter variables and energy balance during the field exercise.

5.2 Hypothesis

1. Isometric leg press and bench press maximal voluntary contraction results decrease during the physically demanding field exercise under negative energy balance and sleep deprivation [Guezennec et al. (1994), Legg et al (1987), Nindl et al. (2007), Nindl et al (2002) and Rognum et al. (1986)].

2. Energy intake is less than energy expenditure during field exercise and body mass decreases [Castellani et al. (2003), Hoyt et al. (2006) and Nindl et al. (2002)].
3. There will be significant changes in hormone concentrations during field exercise – anabolic hormones will decrease and catabolic hormones will increase and possible changes will normalize until the follow measurement. [Adlerceutz et al. (1986), Ishigaki et al. (2005), Karila et al. (2008), Karkoulias et al. (2008), Nindl et al. (2007)].
4. Energy balance relates the physical performance and body mass changes.

6 METHODS

6.1 Subjects

31 men in the end of their military service participated in the study during their intensive training course. Baseline characteristics of the participant population are presented in the table 1. During the measuring period the number of subjects decreased (because of flu or leg injury), and thus the data was analysed from the 26 conscripts.

Table 1. The baseline characteristic of the participant population, (n=31). Maximal aerobic capacity (VO_{2max}) is estimated from the results of 12 minutes Cooper running test and heart rate maximum (HR_{max}) is the highest value during the Cooper running test.

Age (yr.)	**20 ± 1**
VO_{2max} estimated (ml/kg/min)	**53,7 ± 4,5**
HR_{max} (bpm)	**197 ± 9**
Height (cm)	**178,4 ± 7,2**
Body mass (kg)	**71,2 ± 7,2**
Fat percentage (%)	**13,4 ± 4,0**
Fat free mass (kg)	**61,6 ± 6,4**
Fat mass (kg)	**9,6 ± 3,1**
Body mass index	**22,4 ± 1,6**

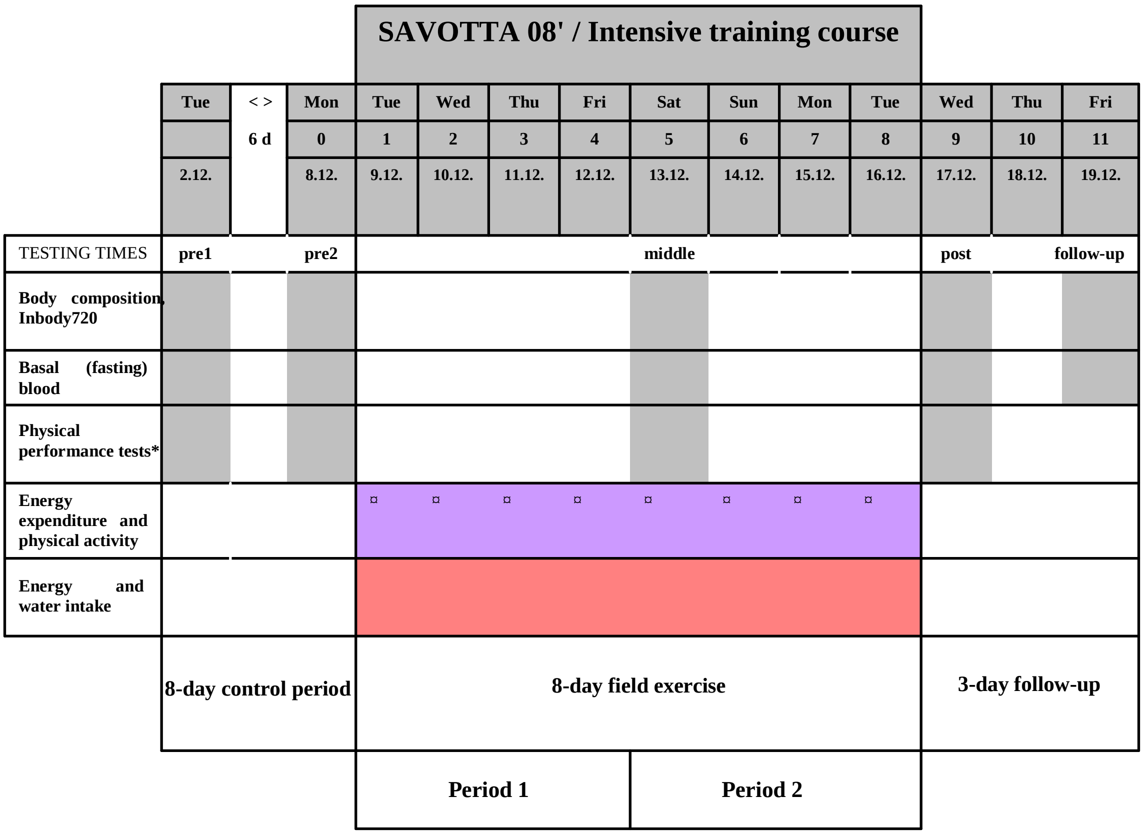
Maximal aerobic capacity was estimated from the results of 12 minutes Cooper running test arranged before actual measuring period. Maximal heart rate was also evaluated from the same running test by using heart rate monitors (Polar S810, RS800). Baseline characteristics in body composition data is derived by using a bioimpedance device (InBody720, Biospace Co. Ltd, Soul, Korea). Stature (cm) was participant's subjective information, and the body mass index was derived from body mass and stature. Body mass index is computed as follows: BMI=Body mass (kg) / stature (m^2). (McArdle et al. p.774.)

6.2 Study design

The Master Thesis is part of the larger study conducted in Kainuu Brigade Singnal Battalion in December 2008. The research data was collected during the SAVOTTA08' field exercise in the Northern Finland. The familiarization measurements were arranged in November 2008 in the Brigade of Kainuu in Kajaani. There was no control group, and the subject population acted as their own controls during control period. The control period is located in the period between pre1 and pre2 measurements. That time was a stable military service time in normal situation in the brigade. After pre2 measurements on 9th of December the participants group started their 8-day field exercise. Post measurements were arranged on the next day in the morning after finishing the field exercise. Follow-up measurements were on the 3rd day in the morning after finishing the field exercise. Pre1, pre2, post and follow-up testing times were all located in the Brigade of Kainuu. The only exception was the measurements during the field exercise (middle tests) which were arranged in the normal school building more near to the locating field exercise region. In this situation the similar performing atmosphere was taken into account.

The timetable (table 2) shows pre1, pre2, middle, post and follow-up measuring dates. In every testing day, body composition, physical performance and basal blood levels were measured besides on the follow-up measurement when no physical performance tests were arranged. Physical performance tests were not suitable for the schedule in the follow-up day. During the 8-day field exercise, the energy expenditure, physical activity, energy and water intake data was collected. The participants kept food diaries while the source of energy consisted of food rations from the Finnish Defence Forces survival package only. The drinking water was melt from the snow. Physical activity data was collected via wrist sensor accelerometer activity meters (Polar WM61, AW200). Energy expenditure was calculated with the information of energy intake and the changes in body energy stores. The changes in body composition were calculated with the information from total body water measurements with deuterium dilution method (Van Marken Lichtenbelt et al. 1994, DeLany et al. 1989).

Table 2. Timetable for the measurement period SAVOTTA08’ field exercise.



				SAVOTTA 08' / Intensive training course										
	Tue	< >	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri
		6 d	0	1	2	3	4	5	6	7	8	9	10	11
	2.12.		8.12.	9.12.	10.12.	11.12.	12.12.	13.12.	14.12.	15.12.	16.12.	17.12.	18.12.	19.12.
TESTING TIMES	pre1		pre2					middle				post		follow-up
Body composition, Inbody720	▨		▨					▨				▨		▨
Basal (fasting) blood	▨		▨					▨				▨		▨
Physical performance tests*	▨		▨					▨				▨		
Energy expenditure and physical activity				¤	¤	¤	¤	¤	¤	¤	¤			
Energy and water intake				🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥			
	8-day control period			**8-day field exercise**								**3-day follow-up**		
				Period 1				**Period 2**						

6.3 Test procedures

6.3.1 Body composition

Body mass was measured five times (pre1, pre2, middle, post, follow-up) by using a bioimpedance device (InBody720 body composition analyzer, Biospace Co. Ltd, Soul, Korea). The measurements were performed between 6:00 and 7:00 a.m. after an overnight fast. Other body composition variables (fat free mass, fat mass, fat percentage) were calculated with the information from total body water measurement by the deuterium dilution technique with standard equations (Schoeller et al. 1986). This technique has been used and validated in soldiers during similar multistressors (DeLany et al. 1989, Nindl et al. 2007). One the latter evening at 10:00 p.m., before the field exercise, subjects were provided a baseline urine sample. After that they ingested a weighed mixture of ^2H_2O. Participants consumed no foods or fluids after dose administration, during the overnight equilibration of isotope with the body water. In the next morning, (field exercise day 1.), subsequent urine samples were collected from the second and third urine voiding. And on the last evening of the field exercise (field exercise day 8.), the process was repeated and urine samples were collected on the next morning after the field exercise. Total body water was calculated as the ^2H dilution space divided by 1.04, correcting for exchange of the ^2H label with nonaqueous H^+ of body solids. (Tanskanen et al. 2012.) Fat free mass (kg) was calculated using the formula: total body water by $^2H_2$ dilution method / 0.73. Fat mass (kg) was calculated subtracting fat free mass (kg) from the body mass (kg).

6.3.2 Basal blood tests /hormones

Basal blood tests were taken on every measuring day (pre1, pre2, middle, post and follow-up) early in the morning when participants were on the fasting state. Serum hormones analysed from the basal blood were total testosterone, cortisol and carrier protein SHBG (sex hormone binding globulin). The blood samples were frozen and stored for further analysing in the laboratory.

6.3.3 Physical performance tests

Warm-up was done in the beginning of the measuring session with cycle ergometer. After cycling participants were advised to do a short stretching. In the middle tests during the 8-day field exercise, there were no possibilities to do warm-up with cycle ergometers and subjects did warm up with jogging approximately the same amount than warm up with cycle ergometer in the Brigade of Kainuu in the pre and post measuring tests.

Bilateral isometric leg extension force and arm extension force, isometric maximal voluntary contraction (IMVC) assessments were performed using the custom-built electromechanical dynamometers (Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland.) In the leg press dynamometer, the subjects were in a sitting position and the knee angle of 107º was individually set to each subject on the familiarization session. Adjustment margin of the measure was checked on the exact point on the every test day. During the performance, the arms were kept crossed on the chest. The back and bottom had to stay on the contact with the seat while pushing. In the bench press dynamometer, the elbow and shoulder angles were 90º and the barbell was placed approximately above sternum. The bottom had to be on the contact with bench press seat and the whole feet had to be on the contact with floor while pushing.

When starting isometric leg and arm extension force measurements, no warm-up trials were done anymore. Participants arrived on the place with pairs, and one seated on the leg press device and another on the bench press device. After adjustments participants were advised to perform pushing as quickly and explosively as they could. Total contraction lasted from three to four seconds ensuring that the maximum contraction was a real maximum and the force level stayed stable. The subjects were verbally encouraged to perform their real maximal effort. Totally three maximal contraction recordings were done with one minute rest between each contraction. After performing three contractions, the pairs changed their testing places. Two best contractions in the leg press and the bench press were used for further analysis.

6.4 Field exercise

SAVOTTA08'-field exercise arranged 9th-16th of December in 2008 in Taivalkoski in the northern Finland. The 8-day field exercise was arranged like war simulated intensive training course. In the military operation soldiers were exposed to sleep deprivation, moderately low energy intake and intermittent intensive physical activity during the field exercise. Daily physical activity consisted of walking, running, cross country skiing, building overnight accommodation and involving all kind of war simulated situations.

The first day, Tuesday, consisted of travelling by bus and preparing the first tasks, scouting and ambush preparation after arriving the district A in the evening at 6 p.m.

The base camp was built at 12 p.m. The participants skied approximately 3km during the first evening and got rest 4 hours during the whole day. The second day started at 4 a.m. by passing eight kilometres to start the preparation of the ambush. The day finished to the 8-hour service break after skiing ten kilometres to the command area. On the third morning, the conscripts company skied 16 kilometres via first and second checkpoints where there was service and first-aid available. The second checkpoint also consisted of 4.5 hours rest. On the fourth day, Friday, passing continued via third and fourth checkpoint to the district B where the company arrived at 4 p.m. after 15 skiing kilometres. The new military tasks started on the action district B. Participants got rest approximately 3 to 5 hours depending on the individual tasks. On the fifth day, Saturday, the military tasks interrupted on those who participated in the study. The participants transported to the Metsäkylä school to take apart and perform the tests related to the study. The tests lasted till 12 a.m., and the participant got back the district B. Preparatory military tasks continued until Sunday morning 12 a.m. when the battle started. Company skied back to the command area and arrived on Monday at 3 a.m. Transportation by skis was 8 to 10 kilometres. The company moved from the command area to the service area, where participants got rest. On the Tuesday morning, the company was transported by buss back to the Kajaani garrison where the post measurements of the study started. The participants skied approximately 70 to 80 kilometres during the field exercise and got rest 28 to 35 hours totally.

6.4.1 Energy and water intake

Participants got their energy from food rations (Finnish Defence Forces survival package). Food ration packs included energy 4215 kcal / 17.87 MJ average per day. The content was 141g from proteins, 612g from carbohydrates and 133g from fats. As energy percentages 14% from proteins, 58% from carbohydrates and 28% from fats. This represents cursorily the recommendations of World Health Organization. Water, which they used, was melted from the snow (in the winter time). Participants kept food diaries during the whole field exercise and marked the amount of water used for drinking and food preparing.

6.4.2 Energy expenditure and physical activity

Physical activity and total rest were assessed using Polar wrist attached sensor accelerometer activity meters (Polar WM61, AW200 Activity Watch, Polar Electro Oy, Kempele, Finland). The function of these activity meters is based on the patented technology and 1-dimension acceleration sensor which counts arm activity but filters the error movements. This activity watch does not include any chest belt to measure heart rate. The AW200 gives the duration of the activity and the active time portion of the total time. This active time is presented as the amount of time spent in four activity zones. (Brugniaux et al. 2008.) Energy expenditure was evaluated with the energy intake and the changes in body energy stores. (Hoyt et al.1999).

The participants worn Polar sensor accelerometer activity meters on their non-dominant wrists the whole time during the 8-day field exercise, from 9th of December at 8 o'clock till 16th of December at 20 o'clock; only exception was the middle measurements (13th of December from 6 o'clock till 10:30 o'clock) while the accelerometers were checked and controlled by the research assistants. All the data from starting the field exercise to the middle of testing day was collected and the same was done after post measurements when the data from the middle of testing day to the end of the field exercise was collected. The time of physical activity was measured in four activity intensity zones: 1) < 1 MET, when person is still, long period of sleeping but also single 30 seconds period being stock-still; 2) 1 - 2 MET, sitting time , typically person is sitting still doing light activities, for example reading, writing, checking a map or eating; 3) 2 – 3.5 MET, standing time, typically person is performing light activities on standing position, on this level person can move short periods (10-20 seconds at time); 4) >3.5 MET, activity time when person is moving and performing continuous motion, >30 seconds or >60 steps per minute.

As a quality assurance, the physical activity was also followed up via the physical activity diaries. In this study the physical activity means military action during the field exercise likewise a variety of military-relevant tasks and skills training, and sustained endurance exercise involving skiing and patrolling activities. During the whole field exercise subjects carried combat gear weighing of 60-70kg inclusive of their clothes and food rations.

6.5 Data analysis

6.5.1 Hormone analysis

For the determination of serum hormone concentrations [total testosterone, cortisol and carrier protein SHBG (sex hormone binding globulin)], 5ml of blood was taken into serum separator tubes and the concentrations were analysed by an immunometric chemiluminescence method with IMMULITE 1000 Analyzer (Siemens Medical Solutions Diagnostics, DPC, Los Angeles, CA, USA) in the laboratory of the Department of Biology of Physical Activity, Jyväskylä Finland. The sensitivities of the assays were 0.5 nmol/L for serum testosterone, 0.2 nmol/L for SHBG and 5.5 nmol/L for cortisol. Reliability (coefficient of variation, CV) for between-day measurements was 8.3% for testosterone, 5.0% for SHBG and 6.1% for cortisol. Intra-assay CV was 5.7% for testosterone, 2.4% for SHBG and 4.6% for cortisol. The serum hormones were analysed from the pre1, pre2, middle, post and follow measurements all in one run. The total hormone concentrations were adjusted with the changes in plasma serum volume.

6.5.2 Bilateral isometric leg and arm extension force (IMVC)

The leg press and bench press devices were connected with the computer via amplifier and D/A converter. All the force data were analysed with Signal 2.16- program. Two of the best performances in leg or bench presses gave the present results for isometric maximum voluntary contraction (F_{max} [N]), maximal rate of force development during 5 ms (RFD_{max} [N/s]) and average force during 0-500 ms [average force in 500ms].

6.5.3 Daily physical activity, energy balance and changes in body composition

Daily physical activity was analysed from the Polar wrist attached sensor accelerometer activity meters (Polar WM61, AW200) by the personnel of Polar. Afterwards, data was arranged by the researcher for further statistical analysing. Like earlier mentioned physical activity was presented in METs, defined as multiples of the resting metabolic rate. One MET is equal for resting oxygen consumption or about 250 ml/min for an average man. Physical activity performed at 2 METs requires twice the resting metabolism, about 500 ml/min for a man, and 3 METs equals three times rest and so on. In more accurate classification considering body size is expressing the MET in terms of oxygen consumption per unit body mass: 1 MET equals 3.5 ml/kg/min. (McArdle et al. 2007, p.203.) In this study we used expression in METs.

Energy intake was analysed from the food diaries with Micro Nutrica 3.0-nutrition calculation program. The exact food consumption information was available for the whole 8-day period because the participants consumed food rations in different paces. The total energy intake during the whole field exercise was divided into eight days getting evaluated total energy intake per day [MJ/day], [kcal/day], [kJ/day/kg] and [kcal/day/kg]. Water consumption is also described per day [L/day] dividing the whole period into eight days. Food content in macronutrients (proteins, carbohydrates and fats) are described as [g/day], [g/kg/day] and Energy %.

Energy expenditure is calculated with the information of energy intake and changes in body composition. Energy expenditure is changes in body energy stores plus energy intake. Changes in body energy stores (changes in fat free mass and fat mass) are calculated via formulas: Changes in fat free mass (FFM) which of it is 27 % protein= Δ protein store (g) x 18.4 kJ and changes on fat mass (FM) = Δ fat mass (g) x 39.8 kJ. (Hoyt et al.1999.) Body composition changes in body mass, fat free mass, fat mass and fat percentage are described as pre and post values (kg) and absolute (kg) and percentage (%) changes. Body mass was measured is taken with InBody on every measuring day.

Energy balance is calculated via the daily energy expenditure [MJ/day] and energy intake [MJ/day]. Water balance is calculated with the information of water loss by deuterium elimination rate, loss of metabolic water and total daily water intake [L/day]. Basal metabolic rate is calculated via formula [BMR = Schofield equation 64.4*weight – 1130*height (m) + 3000 (kJ)].

6.6 Statistical analysis

Standard statistical methods were used for the calculation of means and standard deviations (SD). The differences between measurements and variables were first assessed using a one-way analysis of variance (ANOVA). In cases where there were significant differences, repeated measures were employed using a paired t-test. The $p<0.05$ criterion was used for establishing statistical difference.

Because of there was no control group the subject population acted as their own controls during control period. The control period is located in the period between pre1 and pre2 measurements. In the statistics pre 1 and pre 2 measurements were compared to each other point out the reliability of the stable control period and changes during the measurement period. Possible changes in the main variables were determined comparing the changes to pre 2 measurements if there were no differences between pre 1 and pre 2 measurements. Main variables were the body composition, hormone and physical performance variables. Physical loading, sleeping time, energy intake, energy expenditure and energy balance were assistance variables to understand the main results in the research.

The changes in body composition were shown as an absolute and relative changes between the pre 2 and post measurements and determined if there were statistical changes during the field exercise. The hormone results in the middle, post and follow measurements were first adjusted to plasma serum volume changes comparing to the pre 2 measurement and then calculating the statistical analysis. In the daily physical activity results were divided into two periods. Period 1 was the period from pre 2 measuring day to middle measuring day and period 2 was the period from middle measuring day to post measuring day. In the statistics these two periods are compared to each other on the all activity levels (<1 MET, 1-2METS, 2-3,5METS and <3,5METS). And on every periods the third and fourth activity level were compared to second activity level.

7 RESULTS

7.1 Daily physical activity

The period 1 (from pre2 to middle measurements) referred to a total rest, probably sleep time, < 1MET lasted 233 minutes per night. Soldiers sitting time on the 1-2MET level was 269 minutes, standing time 2-3.5MET level 534 minutes, and active time over 3.5MET level 404 minutes. During period 2 (from middle to post measurements) referred to a total rest < 1MET lasted 303 minutes per night. Soldiers worked on the 1-2MET level 364 minutes probably on sitting position. Standing time on the 2-3.5MET level was 495 minutes, and over 3.5MET level 278 minutes. (Figure 1) On the period 2, all the levels differs significantly from period 1. In the period 1, there were less sleeping and sitting time but more standing and active time.

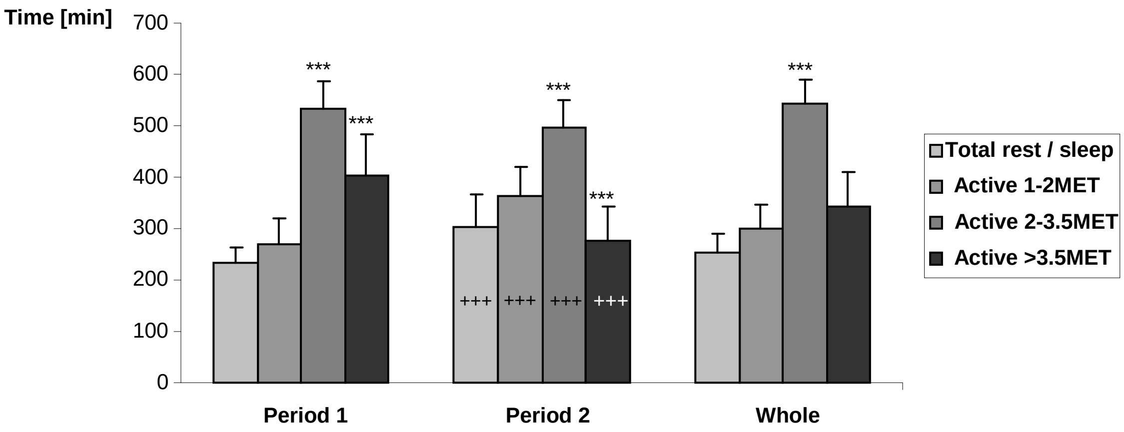


Figure 1. Sleep time and daily physical activity expressed as METs and minutes on each activity level. The field exercise is divided by two periods (period1 is from pre to middle and period 2 is from middle to post measurements). Whole means the whole 8-day field exercise. *** is significant difference compared to Active1-2MET level; +++ (used in period2) significant difference compared to each level on period1, N=24.

7.2 Energy and fluid intake and energy balance

Participants' total energy intake was 12.2±2.7 MJ/day during 8-day field exercise. Considering body size energy intake was 174.1±40.5 kJ/day/kg. In macronutrients carbohydrates intake was 398±73 g/day, in percentages 54±3 E%. Considering body size participants consumed 6±1 g/kg/day carbohydrates. Total fat intake was 92±22 g/day, which means 1±0 g/kg/day considering body mass and in energy percentages fat intake was 29±3 E%. Participants got proteins 123±46 g/day which is as percentages 17±3 E%. Considering body size protein intake was 2±1 g/kg/day. Total water intake was 3.4±0.5 L/day and total water loss was similarly 3.4±0.5 L/day and water deficit was 0.0±0.6 L/day. (Table 3.)

Energy expenditure was approximately 21.9±4.8 MJ/day, the total energy intake 12.2±2.7 MJ/day, and thus the energy balance was -9.7±5.0 MJ/day. Basal metabolic rate was 7334.4±456.1 kJ/day. Physical activity level during field exercise was 3.0±0.7. (Table 4.)

Table 3. Total energy, carbohydrates, fat, protein and fluid intake, average per day divided by eight days (pre-post), N=26.

Total energy intake	
(MJ/day)	**12,2 ± 2,7**
(kJ/day/kg)	**174,1 ± 40,5**
Total carbohydrates intake	
(g/day)	**389 ± 73**
(g/kg/day)	**6 ± 1**
Energy%	**54 ± 3**
Total fat intake	
(g/day)	**92 ± 22**
(g/kg/day)	**1 ± 0**
Energy%	**29 ± 3**
Total protein intake	
(g/day)	**123 ± 46**
(g/kg/day)	**2 ± 1**
Energy%	**17 ± 3**
Total water intake (L/day)	**3,4 ± 0,5**
Total water loss (L/day)	**3,4 ± 0,5**
Water deficit (L/day)	**0,0 ± 0,6**

Table 4. Physical activity level (PAL), Basal metabolic rate (BMR), total energy expenditure (calculated from changes in energy stores plus energy intake), energy intake from food diaries and energy balance. [BMR = Schofield equation 64.4*weight – 1130*height (m) + 3000 (kJ)]. Energy balance = TEE – TEI. N=26.

Physical activity level, PAL	**3,0 ± 0,7**
Basal metabolic rate, BMR (kJ/day)	**7334,4 ± 456,1**
Total energy expenditure (MJ/day)	**21,9 ± 4,8**
Total energy intake (MJ/day)	**12,2 ± 2,7**
Energy balance (MJ/day)	**-9,7 ± 5,0**

7.3 Changes in body composition and relation between energy balance and body composition

Total body mass decreased by 1.8±1kg (p<0.001) during the 8-day field exercise; a relative change was -2.6±1%. Participants' fat free mass slightly increased, but statistically not significantly; absolute change was 0.1±1kg and percentage change 0.2±2%. Fat mass absolute decrease was 2.0±1kg (p<0.001) and a relative change was -18.7±13 %. The absolute change in total fat percentage was -2.4±2% (p<0.001) and a relative change was -16.5±13%. Table 5 shows also pre and post values of each measured variable. Figure 2 describes absolute changes in fat%, fat mass, fat free mass and body mass in pre-post condition.

Table 5. Body mass, fat free mass, fat mass and fat percentage pre and post values and absolute and percentage changes, N=26.

	Body mass (kg)	**Fat free mass** (kg)	**Fat mass** (kg)	**Fat percentage** (%)
pre	**71,4 ± 7**	**60,2 ± 6**	**11,2 ± 3**	**15,6 ± 4**
post	**69,6 ± 7 *****	**60,3 ± 6**	**9,2 ± 3 *****	**13,2 ± 4 *****
change,absolute	**-1,8 ± 1**	**0,1 ± 1**	**-2,0 ± 1**	**-2,4 ± 2**
change (%)	**-2,6 ± 1**	**0,2 ± 2**	**-18,7 ± 13**	**-16,5 ± 13**

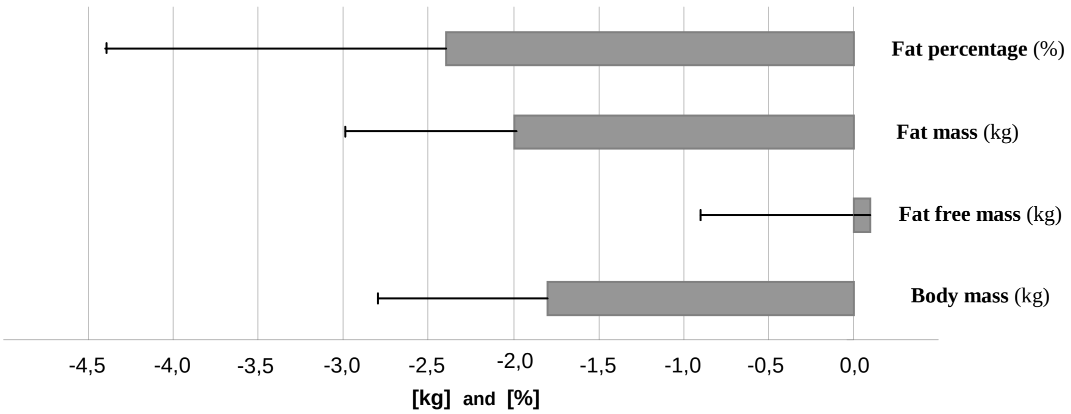


Figure 2. Absolute changes in fat % [%], fat mass, fat free mass and body mass [kg] during the 8-day field exercise (pre-post), N=26.

The whole participant group was divided into three subgroups along with energy balance – low (n=9), mid (n=9) and high (n=8) energy balance groups. Energy balance in group 2 differs significantly compared to group 1 (p<0.001), and group 3 differs significantly compared to group 1 (p<0.001) and group 2 (p<0.001). The changes in body mass are not related with the energy balance in any group but changes in fat mass are related with the energy balance. Fat mass changes in group 2 differs significantly FM changes in group 1 (p<0.001) and FM changes in group 3 differs significantly compared to groups 1 and 2 (p<0.001).

7.4 Changes in physical performance and relation between energy balance and physical performance

Isometric maximum voluntary contraction in bench press was 761±131 N in pre1 measurements and 758±127 N in pre2 measurements. No significant differences between pre1 and pre2 measurements were observed. In the middle measurements, MCV was 676±137 N which differs significantly from pre1 ($p<0.001$), pre2 ($p<0.001$) and post ($p<0.001$) measurements. After the 8-day field exercise MCV was 727±117 N in post measurements which differs significantly from pre1 ($p<0.05$) and pre2 ($p<0.05$) measurements. Maximum voluntary contraction decreased during the field exercise and relative change between pre2 and middle measurements is 11%. (Figure 3.)

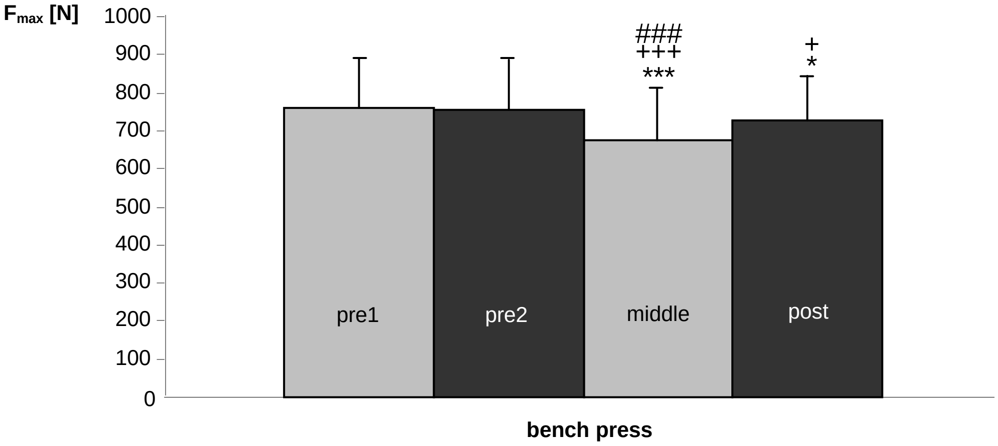


Figure 3. Isometric maximum voluntary contraction in bench press in pre1, pre2, middle and post measurements. *, *** significant difference compared to PRE1-measurement ($p<0.05$, <0.001 respectively); +, +++ significant difference compared to PRE2-measurement; ### significant difference compared to POST-measurement, N=26.

Isometric maximum voluntary contraction in leg press was in 2560±512 N pre1 measurements and 2592±520 N in pre2 measurements. No significant differences between pre1 and pre2 measurements were observed. In the middle measurements, MCV was 2401±584 N which differs significantly from pre1 ($p<0.05$) and pre2 ($p<0.01$) measurements but not from the post measurement. After the 8-day field exercise MCV was 2499±541 N in post measurements which differs from pre2 ($p<0.01$) measurement. Relative change in maximum voluntary contraction between pre2 and middle measurements is 7%. (Figure 4.)

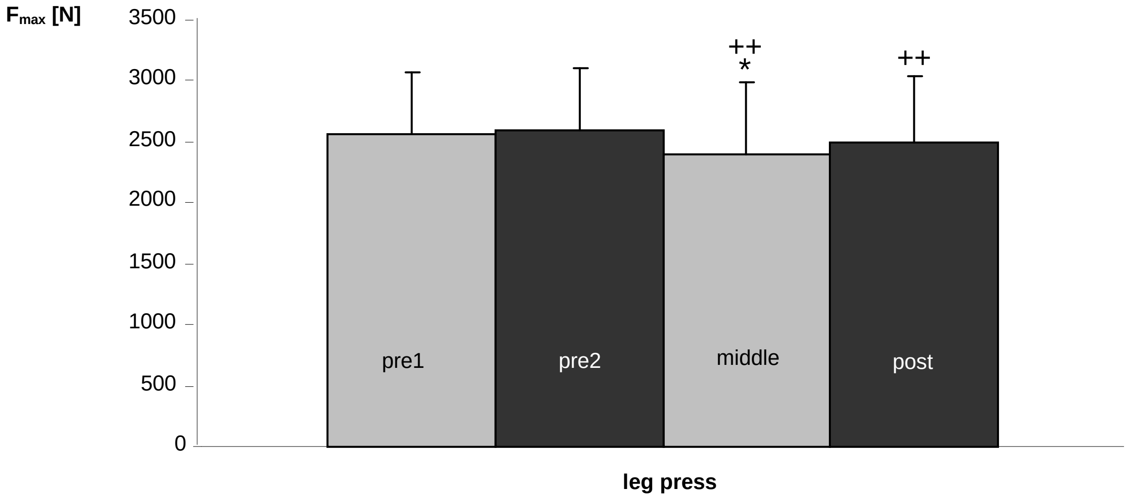


Figure 4. Isometric maximum voluntary contraction in leg press in pre1, pre2, middle and post measurements. *significant difference compared to PRE1-measurement ($p<0.05$); ++ significant difference compared to PRE2-measurement, N=26.

When observing maximal rate of force development (RFD_{max}) in bench press, we can see that RFD_{max} was 24492±12919 N/s in pre1 measurement and 22865±13253 N/s in pre2 measurement. No significant differences between pre1 and pre2 measurements were observed. In the middle measurements maximum rate of force development was 15564±7397 N/s, which differed significantly from pre1 ($p<0.001$) and pre2 ($p<0.01$) measurements. In the post measurements, RFD_{max} was 15942±7276 N/s, which differed significantly from pre1 ($p<0.001$) and pre2 ($p<0.01$) measurements.

Maximal rate of force development in the leg press was 28767±8570 N/s on measuring day pre1 and 25119±8945 N/s in pre2. No significant differences between pre1 and pre2 measurements were observed. In the middle measurements maximal rate of force development in leg press was 16684±7404 N/s, which was significant difference from pre1 ($p<0.001$) and pre2 ($p<0.001$) measurements. In the post measurements RFD_{max} leg press was 16720±7362 N/s, which was significantly different to pre1 ($p<0.001$) and pre2 ($p<0.001$) measurements. (Figure 5.)

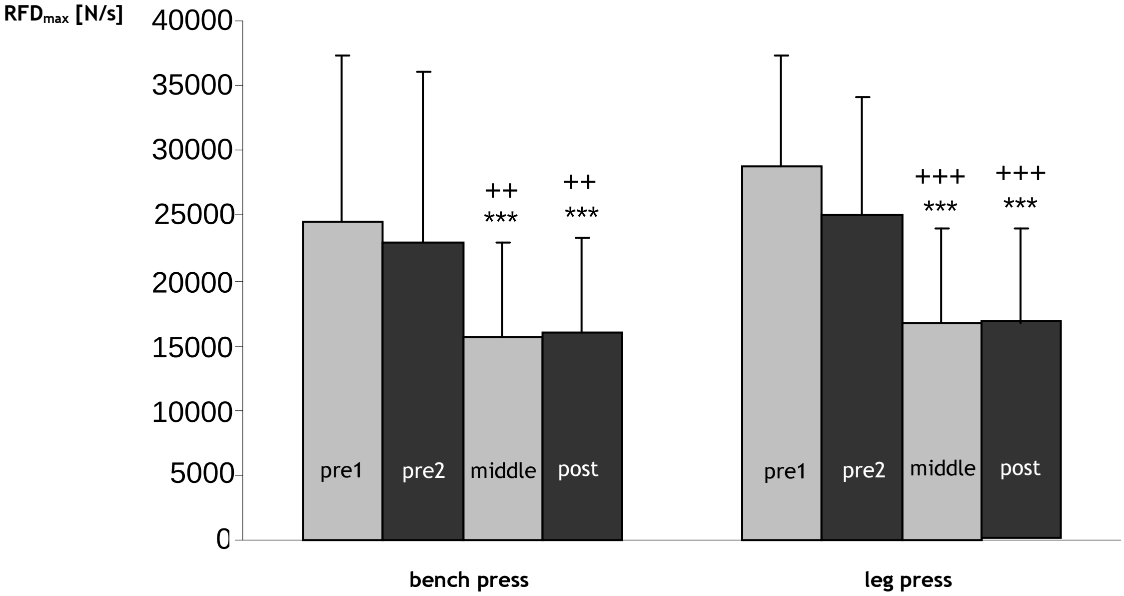


Figure 5. Maximal rate of force development in bench press (left) and leg press (right) in pre1, pre2, middle and post measurements. *** is significant difference compared to PRE1-measurement ($p<0.001$); ++, +++ significant difference compared to PRE2-measurement ($p<0.01$, <0.001 respectively). N=26.

Figure 6 shows an average force production in bench press during 0-500ms. Performance in the middle measurement differed from the other measurements. During the first 100ms there was no significant difference in different measurement days. During 100-200ms the middle measurements differed significantly from pre2 ($p<0.05$) and post ($p<0.05$) measurements. During 200-300ms the middle measurements differed more significantly, compared to pre1 ($p<0.01$), pre2 ($p<0.001$) and post ($p<0.01$) measurements. During 300-400ms the middle measurement differed compared to pre1, pre2 and post, all with the significance ($p<0.001$) and the same situation during 400-500ms (figure 6). There were no significant changes in an average force production in leg press.

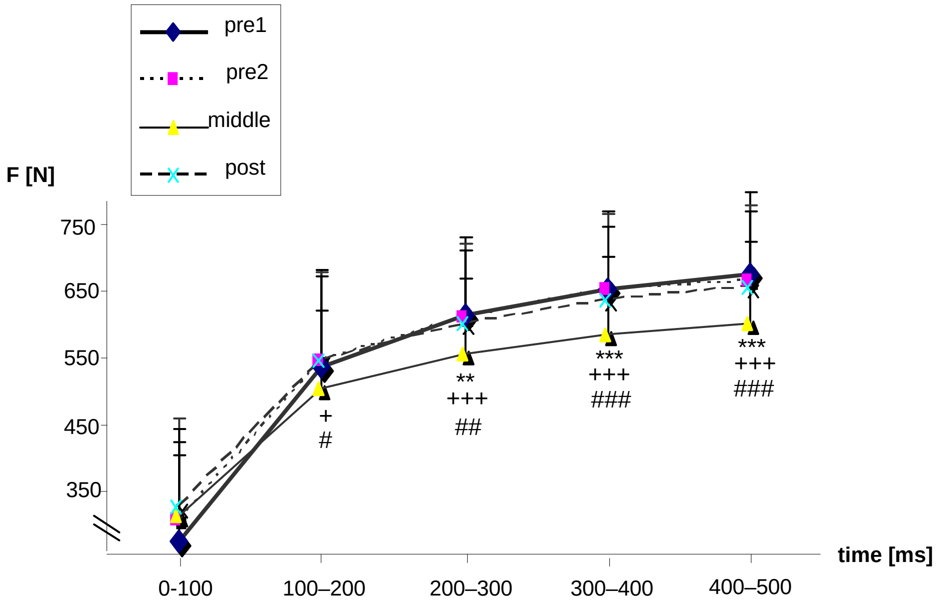


Figure 6. Average force in bench press during 0-500 ms on every measuring day, **, *** significant difference compared to PRE1-measurement ($p<0.01$, <0.001 respectively); +, +++ significant difference compared to PRE2-measurement ($p<0.05$, <0.001 respectively); #, ##, ### significant difference compared to POST-measurement ($p<0.05$, <0.01, <0.001 respectively). N=26.

The same relations than between energy balance and changes in body mass and fat mass were assessed between the energy balance and physical performance. The same groups divided into three subgroups with low, mid and high energy balance differed significantly to each other but the changes in isometric maximal voluntary contraction (nor bench press neither leg press) were not related with the energy balance, but there was a trend.

7.5 Hormones

During the control period the hormonal responses were unchanged. The hormone concentrations in pre 2 measurement did not differ compared to pre 1 measurement in any variables; (470 and 498nmol/L, 21.7 and 21.1nmol/L, 38.4 and 37.1nmol/L, cortisol, testosterone, SHBG respectively). In the hormone concentrations, adjusted to plasma serum volume changes and compared to pre 2 measurements, there was strong significant changes (p<0.001) in all hormone variables in the middle measurements; cortisol increased significantly (648nmol/L), testosterone decreased significantly (14.9nmol/L) and SHBG increased significantly (41.3nmol/L). In the post measurements the cortisol was normalized on the pre2 levels (481nmol/L) but increased slightly but not significantly in the follow measurements (512nmol/L). Testosterone regained slightly from middle to post measurements, increment was significant but it still differed significantly from pre 2 measurement (18.2nmol/L). In the follow measurement the concentration was 22.1nmol/L and it was regained to the pre 2 level and it differed significantly to post measurement. The SHBG concentration increased more after the middle measurement, post and follow measurement levels differed significantly compared to middle measurement (p<0.001). (Figure 7.)

In testo/cortisol-ratio there was a significant decrement in the middle measurement compared to pre 2 measurement (p<0.001) and in the end of the field exercise the T/C-ratio increased to the post measurement and regained even more to the follow measurement; compared to middle measurement a significant difference on both, post and follow measurement (p<0.001). Testo/SHBG-ratio decreased significantly in middle and post measurements compared to pre 2 measurement (both p<0.001), the ratio was lower in the post measurement compared to middle measurement but not significantly. In follow measurement testo/SHBG-ratio increased slightly compared to post measurement (p<0.001) but it did not differ significantly compared to middle measurement. (Figure 8.)

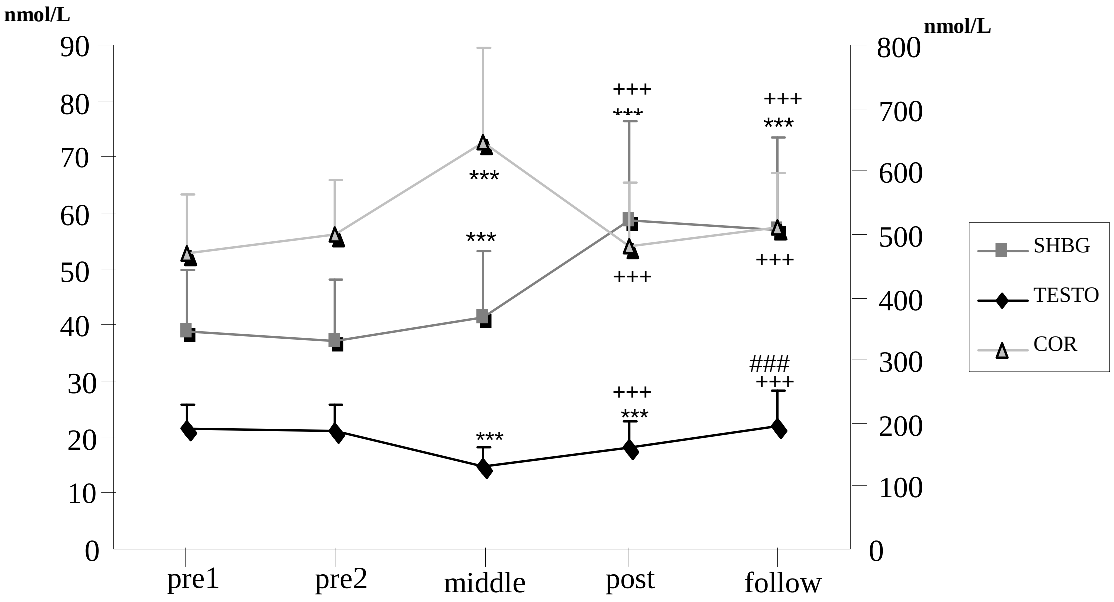


Figure 7. Testosterone & SHBG consentration on the left y-axis and cortisol on the right y-axis. **, *** significant difference compared to PRE2-measurement (p<0.01, <0.001 respectively); +++ significant difference compared to MIDDLE-measurement (p<0.001); ##, ### significant difference compared to POST-measurement (p<0.01 & 0.001). N=25.

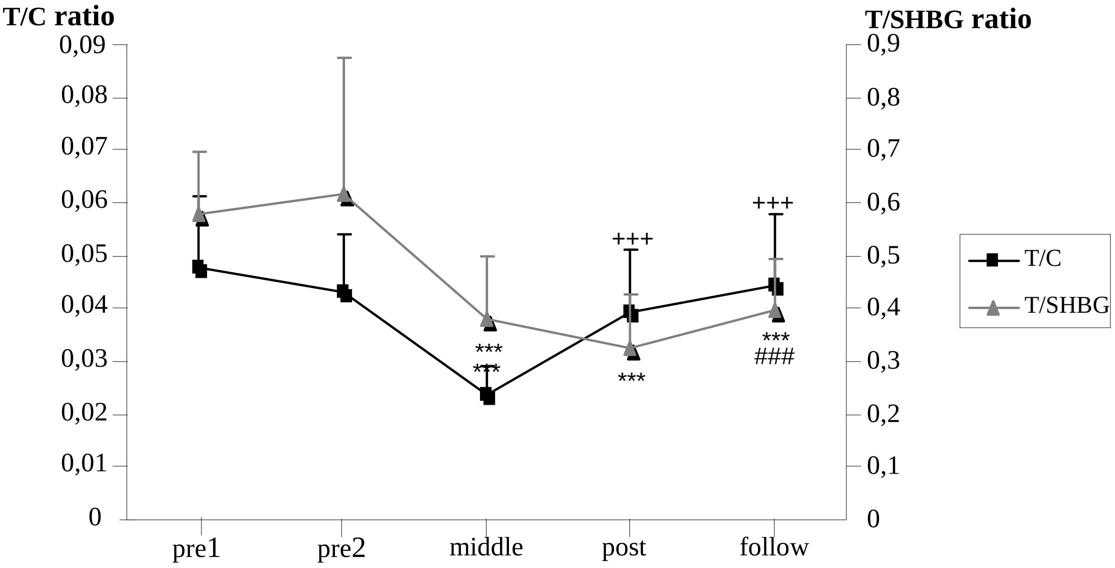


Figure 8. TES/COR-ratio on the left y-axis and TES/SHBG-ratio on the right y-axis. ***significant difference compared to PRE2-measurement (p<0.001); +++ significant difference compared to MIDDLE-measurement (p<0.001); ### significant difference compared to POST-measurement (p<0.01 & 0.001). (N=25).

8 DISCUSSION

The main findings of the present study were significant decrement of bilateral maximum isometric strength both in upper ($p<0.001$) and lower ($p<0.01$) extremities in the middle measurements during the field exercise. 11 and 7% percentage decrement in bench press and leg press respectively makes this result meaningful. The maximal bench press decreased significantly more but on the other hand regained more ($p<0.001$) also to the post measurements in the end of field exercise. Regaining was not significant in leg press in post measurement. The maximal rate of force development in upper and lower extremities decreased also significantly during the field exercise and it did not regain till post measurements in neither bench press nor leg press. The body composition changed during the eight days; body mass and fat mass decreased significantly ($p<0.001$, 1.8±1kg and 2.0±1kg respectively) but lean body mass remained unaltered (slight insignificant decrease). In plasma serum hormones happened changes also; serum cortisol and SHBG concentrations increased significantly ($p<0.001$) and serum total testosterone decreased significantly ($p<0.001$) in the middle measurements compared to pre 2 measurements. In the post measurements the cortisol was normalized on the pre2 levels but it increased slightly (not significantly) in the follow measurements. Testosterone regained slightly from middle to post measurement ($p<0.001$) but it still differed significantly from pre 2 measurement and it also regained from post to follow measurement ($p<0.001$) to the pre 2 levels. The SHBG concentration increased more after the middle measurement, post and follow measurement levels differed significantly compared to middle measurement ($p<0.001$).

In the discussion about the reliability of the methods and results we can speculate that dehydration may affect the body composition results from the InBody720-device. In our study between pre – post measurements total body water did not show any significant changes. Energy balance is calculated via the daily energy expenditure and energy intake. Energy intake was analysed from the food diaries with Micro Nutrica 3.0-nutrition calculation program. Analysing must be done by only one person because of the possible subjective point of view. Energy expenditure was evaluated with the energy intake and the changes in body energy stores so the reliability on the energy expenditure depends on the validity of those variables. Daily physical activity data was analysed from the Polar wrist attached sensor accelerometer activity meters by the personnel of Polar. Accelerometers were reliable to describe the physical loading but were unable to describe the amount of sleep, only the amount of total rest was found. Possible load-carriage was not also found via the accelerometer. Learning effect in leg press and bench press was minimized arranging the pilot 1 measurement before the actual pre 1 and pre 2 measurements. The control period between pre 1 and pre 2 measurements was valid, no significant changes within variables between those time points. For the serum hormone concentrations the blood samples were taken at the same time in the mornings and the samples were frozen and stored for further analysing in the laboratory. Additionally, the hormones were analysed from the all measurements all in one run. The total hormone concentrations were adjusted with the changes in plasma serum volume. So we can speculate the strong reliability of the hormone concentrations.

An individual can maintain high physical activity level and good level of physical performance when she or he has stable energy balance by consuming enough food and nutrients. Many military personnel often fail to match energy intake and energy demands during field training for many reasons. Working almost 20 hours per day there is little time to eat. Many military training scenarios intentionally restrict energy intake to simulate conditions during combat. (Castellani et al. 2006.) Our study also showed that participants involved with negative energy balance while PAL was 3.0. Energy expenditure was 21.9±4.8MJ/day and energy intake 12.2±2.7MJ/day when calculated energy balance was -9.7±5.0MJ/day. In many military studies total energy expenditure has been very high 25-27 MJ/day and participants have worked on very high levels, PAL 3.4±0.5 (Castellani et al. 2006; Gomez-Medino et al. 2002).

Negative energy balance, in our study, conducted the changes in body composition. Body mass decreased 1.8±1kg, fat mass decreased 2.0±1kg and fat percentage -2.4±2% in the end of 8-day field exercise. Many military studies in field training conditions have showed similar results as these in body composition after long or short periods of negative energy balance (Nindl et al. 2007; Hoyt et al. 2006; Castellani et al. 2006 & Nindl et al. 2002).

Significant dehydration which can be seen by blood and urinary parameters do not always occur even if total body water decreases (O’Brien et al. 1996). O’Brien et al.

(1996) showed that the body mass, fat mass and total body water significantly decreased but still the participants had a good hydration state which was seen by blood and urinary parameters. In our study water deficit was 0.0±0.6L when total water intake and total water loss was both 3.4±0.5L.

Body composition changes also have an effect on the serum hormonal changes. Karila et al. (2008) found a significant correlation between reduced weight and decreased serum testosterone concentration. They concluded that even a short-term weight reduction may have a marked effect on body composition, electrolyte homeostasis and hormonal parameters. When the body mass decreases rapidly serum cortisol concentration may increase (Nindl et al. 2007). When body mass or body fat mass decrease the total testosterone concentration may decrease (Nindl et al. 2007; Karila et al. 2008).

In some earlier studies the increase in glucocorticoids during the field exercises is due to a combination physical exercise and energy deficiency. The decrease in testicular androgens might be mainly due to the physical strain since no significant effect was found when cadets were given extra food. (Opstad & Aakvaag, 1983, Adlerceutz et al. 1986.) Guezennec et al. (1994) also concluded that extra food might reduce the decrement of testosterone during a military training course. Earlier studies have shown that endurance type training affects serum hormone concentrations, decreasing testosterone and increasing cortisol (Brownlee, 2005; Väänänen, 2002; Fry et al. 1998; Lucia et al. 2001; Brownlee et al. 2005). In our study the participants were also influenced under caloric deprivation and weight loss nonetheless subjects also influenced more endurance type of training during the field exercise.

Sleep deprivation may not have an effect on hormone concentrations. Abedelmalek (2013) and Martin et al. (1986) concluded that sleep loss did not alter the stress hormonal response (cortisol) during subsequent exercise. Remes et al (1985) resulted that the sleep deprivation did not cause significant changes in the mean plasma SHBG or T/SHBG ratio. A slower decrease in testosterone happened when participants was given three hours of extra sleep each night. (Remes et al. 1985). Gomez-Medino et al. (2002) determined significantly decreased testosterone during 5-d military training exercise but during the field exercise participants were also under energy restriction and physical strain.

However sleep deprivation might be the part of decrement in physical performance. Legg et al. (1987) and Rognum et al. (1986) resulted that the major factor influencing performance and well-being in these experiments was sleep deprivation rather than the sustained physical activity. It is also concluded that the decline in performance could not be prevented by giving a high-energy diet alone. In conclusion we can emphasize how important even brief periods of sleeps are. Still numerous studies have shown no effects on physical performance. Sleep deprivation of 30 to 72 hours does not affect muscle strength or electromechanical responses, but time to exhaustion according to VanHelder et al. (1989). Sleep deprivation might have an effect on the arousal stage and responding in muscle activation which result the decrement in rate of force development. Whereas Blumert et al. (2007) resulted that 1RM weight lifting performance did not alter after sleep deprivation. Symons et al. (1988) suggest that sleep loss of up to 60 hours will not impair the capability for physical work because maximal isometric and isokinetic muscular strength and endurance of selected upper and lower body muscle groups were not significantly altered. Haslam (1984) concluded that the effects of sleep loss are psychological rather than physiological. Many authors speculate that the possible decrease in performance might be due to a decrease in arousal and diminished motivation.

Physical loading itself may not influence a lot to physical performance even if it is not enough stressful. Rissanen et al. (2005) have resulted that maximal force of leg extensors do not change during prolonged 12-day military operation. Rate of force development decreased. Väänänen et al. (2001 & 1997) also showed no significant changes in the functional muscle strength of the lower limb during ultra-long exercise (165km march, ~250 000 steps, 10-kg backpack, water + energy freely available). We must still speculate that prolonged endurance type of training may reduce neuromuscular function and this could have an effect on physical performance in our measurements. Maximal voluntary contraction and maximal rate of force development hindered between the period 1. Maximum strength regained slightly but maximal rate of force development remained at the end of period 2. This hindered level of maximal rate of force development still in the post measurement might be due to high physical loading and sleep deprivation.

Even if earlier studies concluded that sleep deprivation has a minimal effect on physical performance, we have to emphasize the synergy of all factors during the field exercise. We have to be careful in speculating because we do not know the exact amount of sleep during the field exercise – we only know the amount of total rest which was 233 minutes and 303 minutes during the period 1 and the period 2, respectively.

In the study of Nindl et al. (2007), body composition changes affected the serum hormone changes and those together affected in the changes in physical performance. These results support also our study where the results showed body composition changes and serum hormonal changes and physical performance decrement. Friedl & Hoyt (1997) suggested in their review that body mass losses of at least 5-10% are necessary before any significant decrements in performance occur. In our study the percentage change in body mass was only -2.6±1% during the field exercise. In other words only body mass decrement did not affect the hindered physical performance.

In our study the significantly hindered testo/corticol-ratio and testo/SHBG-ratio in middle measurement support the hypothesis that physical loading and sleep deprivation or lack of rest was enough strenuous to affect also the physical performance during the field exercise. The isometric voluntary contraction was lowered in the middle measurements in bench press and leg press. Interestingly testo/cortisol-ratio was not anymore significantly hindered level in the post or follow measurements and neither were maximal strength in bench press. In leg press it was still lowered. The lower extremities were maybe under more exhaustive loading than upper extremities during the field exercise because of skiing.

9 CONCLUSIONS

It is difficult to point how much the different exposures have impact on the findings. The participants worked eight days under sleep deprivation and negative energy balance and they were highly physically active. All of these factors influence on body composition, serum hormones and physical performance along or apart. Negative energy balance and changes in body mass affects hormonal parameters. That influenced the body composition changes which affected on the serum hormonal changes and those together had an effect on physical performance changes. Even if in our findings negative energy balance did not straight correlate to physical performance. The endurance type physical loading might also have a strong effect on physical performance in the results. Nonetheless the physical activity during the field exercise was not so high than in the other studies, 3.0 versus 3.5.

Muscular strength is an essential component for optimal military performance. For that reason optimal situation would be to keep the muscular strength on the good level also during the war simulated field exercises. Maintenance in muscular strength may be facilitated by the energy balance and adequate nocturnal sleep or several short naps during the day during prolonged field exercise.

10 ACKNOWLEDGEMENTS

I want to equally thank my supervisors Heikki Kyröläinen and Keijo Häkkinen, and specially Minna Tanskanen to let me work under the SAVOTTA08'- field exercise and the measurements which were mainly for her Doctoral studies. I want to also thank Elina Kokkonen for her assistance in the data analysis, Risto Puurtinen for his assistance in the hormone analysis and Susanne Kumpulainen and Heidi Syväoja for their assistance in the measurements during the field exercise.

11 REFERENCES

Abedelmalek, S., Souissi, N., Chtourou, H., Denquezli, M., Aouichaoui, C., Ajina, M., Aloui, A., Digui, M., Haddouk, S. & Tabka, Z. (2013). Effects of Partial Sleep Deprivation on Proinflammatory Cytokines, Growth Hormone, and Steroid Homone Concentrations, During Repeated Briedf Sprint Inteval Exercise. Chronobiology international 30(4):502-509.

Achten, J. & Jeukendrup, A., (2003). Heart rate monitoring: applications and limitations. Sports Medicine. 33(7):517-538.

Bouchard C., Blair S.N. & Haskell W.L. (2007). Physical Activity and Health. Champaign: Human Kinetics.

Brugniaux, J., Niva, A., Pulkkinen, I., Laukkanen, R., Richalet J.-P. & Pichon A. (2010). Polar Activity Watch 200: a new device to accurately assess energy expenditure. British Journal of Sports Medicine. 44: 245-249.

Burstein, R., Coward, A., Askew, W., Carmel, K., Irving, C., Shpilberg, O., Moran, D., Pikarsky, A., Ginot, G., Sawyer, M., Golan, R. & Epstein, Y. (1996). Energy expenditure variations in soldiers performing military activities under cold and hot climate conditions. Military Medicine **161(12): 750-4.**

Castellani, J., Stulz, D., DeGroot , D., Blanchard, L., Cadaette, B., Nindl, B. & Montain, S. (2003). Eighty-four hours of sustained operations alter thermoregulation during cold exposure. Medicine & Science in Sports and Exercise. 38(5): 894-900.

Castellani, J., Delany, J., O'Brien, C., Hoyt, R., Santee, W. & Young, A. (2006). Energy expenditure in men and women during 54 h of exercise and caloric deprivation. Medicine & Science in Sports and Exercise 38(5): 894-900.

Crewther, B., Keogh, J., Cronin, J. & Cook, C., (2006). Possible stimuli for strength an power adaptation: acute hormonal responses. Sports Medicine. 36(3):215-238.

Daly, W., Seegers, C., Rubin, D., Dobridge, J. & Hackney, A. (2005). Relationship between stress hormones and testosterone with prolonged endurance exercise. European Journal of Applied Physiology. 93(4): 375-80.

DeLany, J., P., Schoeller, D., A., Hoyt, R.,W., Askew, E., W. & Sharp, M., A., (1989). Field use of $D_2{}^{18}O$ to measure energy expenditure of soldiers at different energy intakes. Journal of Applied Physiology. 67:922-1929.

Fogelholm, M., Koskinen, R., Laakso, J., Rankinen, T. & Ruokonen, I. (1993) Gradual and rapid weight loss: effects on nutrition and performance in male atheletes. Med. Sci. Sports Exerc. 25(3): 371-377.

Friedl, K. & Hoyt, R. (1997). Development and biomedical testing of military operational rations. Annual Review of Nutrition 17:51-75.

Fry, A., Kraemer, W. & Ramsey, L. (1998). Pituitary-adrenal-gonadal responses to high-intensity resistance exercise overtraining. Journal of Applied Physiology 85: 2352–2359.

Giam, G. (1997). Effects of sleep deprivation with reference to military operations. Annals Academy Medicine Singapore. 26(1): 88-93.

Gomez-Merino, D., Chennaoui, M., Burnat, P., Crogou, C. & Guezennec, C. (2003). Immune and hormonal changes following intense military training. Military Medicine 168: 1034-8.

Guezennec, C., Satabin, P., Legrand, H. & Bigard, A. (1994). Physical performance and metabolic changes induced by combined prolonged exercise and different energy intakes in humans. European Journal of Applied Physiology. 68:525-530.

Guglielmini, C., Paolini, A.R. & Conconi, F. (1984) Variations of Serum Testosterone Concentrations after Physical Exercise Of Different Duration. International Journal of Sports Medicine 5(5): 246-249.

Haslam, D.R. (1984). The military performance of soldiers in sustained operations. Aviation Space and Environmental Medicine, 55(3), 216-221.

Hoyt, R., Jones, T., Stein, T., McAninch, G., Lieberman, H., Askew, E. & Cymerman, A. (1991). Doubly labeled water measurement of human energy expenditure during strenuous exercise. J Appl Physiol. 71(1):16-22.

Hoyt, R., Opstad, P., Haugen, A.-H., Delany, J., Cymerman, A. & Friedl, K. (2006). Negative energy balance in male and female rangers: effects of 7 d of sustained exercise and food deprivation. American Journal of Clinical Nutrition. 83:1068-1075.

Huovinen, H., Hulmi, J., Isolehto, J., Kyröläinen, H., Puurtinen, R., Karila, T., Mackala, K. & Mero, A. (2015). Body composition and power performance improved after weight reduction in male athletes without hampering hormonal balance. The Journal of Strenght and Corresponding Research 29(1):29-36.

Häkkinen, K., Pakarinen, A., Alén, M., Kauhanen, H. & Komi, P. (1988). Neuromuscular and hormonal responses in elite athletes to two successive strength training sessions in one day. European Journal of Applied Physiology and Occupational Physiology. 57(2):133-9.

Ishigaki, T., Koyama, K., Tsujita, J., Tanaka, N., Hori, S. & Oku, Y. (2005) Plasma Leptin Levels of Elite Endurance Runners after Heavy Endurance Training. Journal of Physiological Anthropology and Applied Human Science 24(6): 573-578.

Karila, T., Sarkkinen, P., Marttinen, M., Seppälä, T., Mero, A. & Tallroth, K.(2008). Rapid weight loss decreases seum testosterone. International Journal of Sports Medicine 29:872-877.

Karkoulias, K., Habeos, I., Charokopos, N., Tsiamita, M., Mazarakis, A., Poili, A. & Spiropoulos, K. (2008). Hormonal responses to marathon running in non-elite athletes. European Journal of Internal Medicine 19(8): 598-601.

Kraemer, W., Patton, J., Gordon, S., Harman, E, Deschenes, M., Reynolds, K., Newton, R., Triplett, N. & Dziados, J. (1995). Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations. Journal of Applied Physiology 78(3):976-89.

Kraemer, W., Loebel, C., Volek, J., Ratamess, N., Newton, R., Wickham, R., Gotshalk, L., Duncan, N., Mazzetti, S., Gomez, A., Rubin, M., Nindl, B. & Hakkinen, K. (2001). The effect of heavy resistance exercise on the circadian rhythm of salivary testosterone in men. European Journal of Applied Physiology 84:13 –18.

Kyröläinen, H., Oksanen, H., Finni, T., Vaara, J., Koivu, M. & Virmavirta M. (2007). Effects of sleep deprivation on neuromuscular function. Journal of Biomechanics 40(2): s419.

Legg, S. & Patton, J. (1987) Effects of sustained manual work and partial sleep deprivation on muscular strength and endurance. Eur J Appl Physiol 56:64-68.

Lucia, A., Diaz, B., Hoyos, J., Fernandez, C., Villa, G., Bandres, F. & Chicharro, J. (2001). Hormone levels of world class cyclists during the Tour of Spain stage race. British Journal of Sports Medicine 35: 424–430.

Lyons, J., Allsopp, A. & Bilzon, J. (2005). Influences of body composition upon the relative metabolic and cardiovascular demands of load-carriage. Occupational Medicine 55: 380-384.

Martin, B., Bender, P. & Chen, H. (1986). Stress hormonal response to exercise after sleep loss. European Journal of Applied Physiology and Occupational Physiology 55(2):210-4.

Martin, B. (1981). Effect of sleep deprivation on tolerance of prolonged exercise. European Journal of Applied Physiology and Occupational Physiology 47(4):345-54.

McArdle, W., D., Katch, F., I. & Katch, V., L. (2007). 6th Edition. Exercise Physiology – Energy, Nutrition & Human Performance. Lippincott Williams & Wilkins. ISBN 0-7817-4990-5.

McCaig, R. & Gooderson, C. (1986). Ergonomics and physiological aspects of military operations in cold wet climate. Ergonomics. 29(7): 849-857.
Morton, R., Fitz-Clarke, J. & Banister, E. (1990). Modeling human performance in running. Journal of Applied Physiology 69(3):1171-1177.

Nindl, B., Leone, C., Tharion, W., Johnson, R., Castellani, J., Patton, J. & Montain, S. (2002). Physical performance responses during 72 h of military operational stress. Medicine and Science of Sports Exercise 34(11): 1814–1822.

Nindl, B., Barnes, B., Alemany, J., Frykman, P., Shippee, R. & Friedl, K. (2007). Physiological Consequences of U.S. Army Ranger Training. Medicine and Science of Sport Exercise 39(8): 1380-1387.

O'Brien, C., Freund, B., Sawka, M., McKay, J., Hesslink, R. & Jones T. (1996). Hydration assessment during cold-weather military field training exercises. Arctic Medical Research 55(1):20-6.

Opstad, P. (1995). Medical consequences in young men of prolonged physical stress with sleep and energy deficiency. Norwegian Defence Research Establishment publication -95/05586.

Opstad, P. (1994). Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. European Journal of Endocrinology 131(1):56-66.

Opstad, P. & Aakvaag, A. (1983). The effect of sleep deprivation on the plasma levels of hormones during prolonged physical strain and calorie deficiency. European Journal of Applied Physiology and Occupational Physiology 51(1): 97-107.
Rissanen, S., Oksa, J., Kyröläinen, H., Mäkinen, T., Santtila, M., Mäntysaari, M., Rintamäki, H. and Peitso, A. (2005) An abstract: Effects of 12 days military winter operation on neuromuscular performance. In Congress proceedings: International Congress on Soldiers' Physical Performance. May 18-22, 2005, Jyväskylä, Finland.

Rognum, T., Vartdal, F., Rodahl, K., Opstad, P., Knudsen-Baas, O., Kindt, E. Withey, W. (1986). Physical and mental performance of soldiers on high- and low-energy diets during prolonged heavy exercise combined with sleep deprivation. Ergonomics 29(7):859-867.

Schiotz, M., Potteiger, J., Huntsinger, P., & Donald, D. (1998) The Short-Term Effects of Periodized and Constant-Intensity Training on Body Composition, Strength, and Performance. Journal of Strength and Conditioning Research 12(3):173-178.

Schoeller, D., Ravussin, E., Schutz, Y., Acheson, K., Baertschi, P. & Jequier, E. (1986). Energy expenditure by doubly labelled water; validation in humans and proposed calculation. American Journal of Physiology. 250:R823-R830.

Sgrò P1, Romanelli F, Felici F, Sansone M, Bianchini S, Buzzachera CF, Baldari C, Guidetti L, Pigozzi F, Lenzi A, Di Luigi L.(2014). Testosterone responses to standardized short-term sub-maximal and maximal endurance exercises: issues on the dynamic adaptive role of the hypothalamic-pituitary-testicular axis. Journal of Endocrinological Investigations. 37(1):13-24

Symons, J., Vanhelder, T. & Myles, W. (1988). Physical performance and physiological responses following 60 hours of sleep deprivation. Medicine &. Science of Sports Exercise 20(4): 374-380.

Tanskanen, M., Westerterp, K., Uusitalo, A., Atalay, M., Häkkinen, K., Kinnunen, H. and Kyröläinen, H., (2012). Effects of Easy-To-Use Protein-Rich Energy Bar on Energy

Balance, Physical Activity and Performance during 8 Days off Sustained Physical Exertion. PLoS One 7(10):e47771.

Vaara, J., Kyröläinen, H., Koivu, M., Tulppo, M. & Finni, T. (2009) The effect of 60-h sleep deprivation on cardiovascular regulation and body temperature. European Journal of Applied Physiology 105(3):439-44.

VanHelder, T. & Radomski, M. (1989). Sleep Deprivation and the Effect on Exercise Performance. Sports Medicine 7(4):235-247.

Van Marken Lichtenbelt, Westerterp, K & Wouters, R. (1994) Deuterium dilution as a method for determining total body water: effect of test protocol and sampling time. British Journal of Nutrition 72(4): 491-7.

Vingren, J., Kraemer, W., Ratamess, N., Anderson, J., Volek, J. & Maresh, C. (2010). Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. Sports Medicine 40(12):1037-53.

Väänänen, I., Mäntysaari, M. & Huttunen, P. (1997) The Effects of a 4-Day March on the on the Lower Extremities and Hormonal Balance. Military Medicine 162(2): 118-122.

Väänänen, I., Mäntysaari, M. & Vihko, V. (2001) Soldiers' physiological and psychological loading during a 4-day march. Annales Medicinae Militaris Fenniae 76(1): 59–67.

Väänänen, I., Vasankari, T., Mäntysaari, M. & Vihko, V. (2002). Hormonal responses to daily strenuous walking during 4 successive days. European Journal of Applied Physiology 88(1-2):122-7

Väänänen, I., Vasankari, T., Mäntysaari, M. & Vihko, V. (2004). Hormonal responses to 100 km cross-country skiing during 2 days. Journal of Sports Medicine and Physical Fitness 44(3):309-14.

Väänänen, I. & Vihko, V. (2005). Physiological and psychological responses to 100 km cross-country skiing during 2 days. Journal of Sports Medicine and Physical Fitness 45(3):301-5

Walker, B., Best, R., Noon, J., Watt, G. & Webb, D. (1997). Seasonal Variation in Glucocorticoid Activity in Healthy Men. Journal of Clinical Endocrinology and Metabolism 82(12): 4015-4019.

Young, A., Castellani, J., O'Brien, C., Shippee, R., Tikuisis, P., Meyer, L., Blanchard, L., Kain, J., Cadarette, B. & Sawka, M. (1998). Exertional fatigue, sleep loss, and negative energy balance increase susceptibility to hypothermia. Journal of Applied Physiology 85(4): 1210–1217.

Zachwieja, J., Ezell, D., Cline, A., Ricketts, J., Vicknair, P., Schole, S. & Ryan, D. (2001) Short-term dietary energy restriction reduces lean body mass but not performance in physically active men and women. International Journal of Sports Medicine 22(4): 310-16.